

# Calmodulin Triggers the Resumption of Meiosis in Amphibian Oocytes

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**ABSTRACT** The calcium-binding protein, calmodulin, has been purified from *Xenopus laevis* oocytes. This 18,500-dalton protein, pI 4.3, has two high-affinity calcium-binding sites per mole protein having a dissociation constant of  $2.8 \times 10^{-6}$  M.

Full-grown *Xenopus* oocytes, arrested in late G<sub>2</sub> of the meiotic cell cycle, resumed meiosis when microinjected with 60–80 ng (3–4 pmol) of calmodulin in the form of a calcium-calmodulin complex. The timing of the meiotic events in these recipient oocytes was the same as that normally induced by progesterone.

*Xenopus* ovarian calmodulin stimulated bovine brain phosphodiesterase (PDE) 3- to 10-fold in a calcium-dependent manner, but it had no apparent effect on ovarian PDE activity. A calcium-calmodulin-dependent protein kinase has been isolated from *Xenopus* oocytes using a calmodulin-Sepharose 4B affinity column. The possible role for this kinase in regulating the G<sub>2</sub>-M transition in oocytes has been discussed.

Amphibian oocytes, arrested in late prophase of meiosis (G<sub>2</sub>), resume meiosis in response to progesterone (26, 31). This resumption leads to the dissolution of the nuclear membrane, followed by chromosome condensation and the meiotic divisions up to the second metaphase. Several observations have led to the suggestion that a progesterone-induced increase in intracellular free calcium may be a necessary first step for the resumption of meiosis (1, 15, 17, 30). Recently, we have demonstrated directly, using the calcium-specific photoprotein aequorin, that progesterone does cause a rapid, but only transient increase in the free calcium level in oocytes (32). The mechanism by which an increased calcium activity might regulate the G<sub>2</sub>-M transition remains unsolved at this time.

Increasing evidence indicates that a calcium-binding protein, designated calmodulin, plays a key role in regulating many calcium-dependent processes. Calmodulin is a highly conserved protein that has been identified in a variety of cell types; we have presented preliminary evidence suggesting that *Xenopus* oocytes also contain this protein (27). In the current study, we have more fully characterized oocyte calmodulin with respect to its physical and biological properties. More significantly, we demonstrate that oocyte calmodulin, in the form of a calcium-calmodulin complex, can trigger the resumption of

meiosis (oocyte maturation) when injected into full-grown *Xenopus* oocytes.

We have attempted to elucidate the mechanism by which calmodulin induces the resumption of meiosis in oocytes. The results indicate that oocyte calmodulin does not regulate ovarian phosphodiesterase but may be involved in the activation of oocyte protein kinases.

## MATERIALS AND METHODS

### *Isolation of Calmodulin from Xenopus Oocytes and Bovine Brain*

Approximately 75 g (wet wt) of *Xenopus* ovary were processed as described by Dedman et al. (5) for the preparation of rat testis calmodulin, with the following minor modifications: Ovarian cytosol was heated at 85°C for only 3 min instead of 5 min. Fractions eluting off the DE-52 column between 0.25 and 0.35 M NaCl contained ovarian calmodulin. After dialysis and lyophilization, the protein sample was then applied to a Sephadex G-75 column. Fractions containing calmodulin were pooled, dialyzed, lyophilized, and stored at –20°C.

A second procedure of Watterson et al. (33), used to isolate calmodulin from bovine brain, was also used to prepare calmodulin from *Xenopus* oocytes. Ovarian calmodulin precipitated out of homogenates at between 55 and 65% ammonium sulfate saturation at pH 4.1. The protein sample was then applied to a DEAE-Sephadex A-50 column. Fractions eluting off the column between 0.5 and 0.6 M NaCl were pooled, dialyzed, lyophilized, and subjected to gel filtration. All buffers were the same as those described by Dedman et al. (5) and by Watterson

<sup>1</sup> Wasserman and Smith. Unpublished data.

et al. (33). Approximately 500 g (wet wt) of fresh bovine brain (less membranes and spinal cord) were processed by the procedures of Watterson et al. (33).

## Isolation of cAMP and cGMP Phosphodiesterases from *Xenopus* Oocytes and Bovine Brain

Two *Xenopus* ovaries were dispersed into individual defolliculated oocytes using 0.15% collagenase type II (Sigma Chemical Co., St. Louis, Mo.) in Ca-free OR-2 medium (29). Released oocytes were washed in 0.05 M  $\beta$ -glycerophosphate, 0.15 NaCl, 0.001 M  $\beta$ -mercaptoethanol, 0.001 M EDTA, and 0.25 M sucrose, pH 7.2, and then packed in this buffer under 1 g in a centrifuge tube. The oocytes were crushed at 25,000 g for 15 min and then the supernate was subjected to ammonium sulfate precipitation. Bovine brain tissue was processed as described by Watterson et al. (33). Phosphodiesterase (PDE) activity precipitated out of supernates at between 30 and 55% ammonium sulfate saturation. The ovary or brain samples were then loaded on a DEAE-Sephadex A-50 column and eluted off with an NaCl gradient (0.2–0.8 M). Fractions containing the majority of PDE activity were pooled, dialyzed, and lyophilized. These partially purified PDE preparations were stored at  $-20^{\circ}\text{C}$ .

### Physical Characterization of Calmodulin

**MOLECULAR WEIGHT AND PI DETERMINATION:** The molecular weight of ovarian calmodulin was determined by gel filtration through Sephadex G-75 and by SDS polyacrylamide gel electrophoresis as described earlier (27). Gel electrofocusing of *Xenopus* ovarian calmodulin was also carried out as described earlier (27).

**CALCIUM-BINDING PROPERTIES OF OVARIAN CALMODULIN:** The calcium dissociation constant of ovarian calmodulin was determined by equilibrium dialysis using  $^{45}\text{Ca}/\text{EGTA}$  buffers as described by Dedman et al. (5). Desired free  $\text{Ca}^{2+}$  concentrations, in equilibrium with EGTA, were obtained by adding known amounts of unlabeled  $\text{Ca}(\text{OH})_2$  according to the calculations of Rafflaub (21). The  $\text{Ca}^{2+}$ -binding constant of EGTA was corrected for pH 7.0. Dialysis was carried out for 48 h at  $2^{\circ}\text{C}$  and the amount of  $\text{Ca}^{2+}$  bound to calmodulin was then determined.

### Biological Properties of Calmodulin

**MICROINJECTION OF THE CALCIUM-CALMODULIN COMPLEX:** Oocyte calmodulin was dissolved in 40 mM Tris-HCl, 3 mM  $\text{MgCl}_2$ , 0.01 mM  $\text{CaCl}_2$ , pH 8.0 buffer, at 2 mg/ml. The calcium-calmodulin complex was microinjected into oocytes with a multiple injection micropipette (14). Oocytes were scored for nuclear membrane dissolution ( $\text{G}_2\text{-M}$  transition) by the method described elsewhere (30).

### Effect of Ovarian Calmodulin on cAMP and cGMP PDE Activities

PDE activity was assayed by monitoring the conversion of 3',5'-cAMP or 3',5'-cGMP to 5'-AMP or 5'-GMP, respectively, by a two-step method. The product of the reaction, 5'-AMP, or 5'-GMP, was converted to adenosine or guanosine and inorganic phosphate by incubation with 5'-nucleotidase. The released phosphate then was determined colorimetrically by the method of Sanui (23).

### Calmodulin Affinity Column

Approximately 10 mg of oocyte calmodulin was coupled to CNBr-activated Sepharose 4B (Pharmacia) by the procedure of Klee and Krinks (7). Protein samples were dissolved in a calcium-binding buffer that contained 40 mM Tris-HCl, pH 7.5, 50 mM NaCl, 0.2 mM  $\text{CaCl}_2$ , and 3 mM  $\text{MgCl}_2$ , and were dialyzed against this buffer overnight. 5 mg of protein was passed through the affinity column in the calcium-binding buffer. After the unbound protein was washed off, the solution was changed to an EGTA-containing buffer (2 mM) and the "bound" protein fraction was eluted off with the EGTA buffer front. Calcium-calmodulin-dependent protein kinase activity was assayed by the procedures of Schulman and Greengard (24).

## RESULTS

### Physical Properties of Ovarian Calmodulin

The physical characteristics of oocyte calmodulin are summarized in Table I which also includes comparable data ob-

tained from the literature for calmodulin isolated from various sources. It is evident that oocyte calmodulin is very similar if not identical to the protein from other tissues. The one potential exception to this statement may involve the number of high-affinity calcium-binding sites that exist on oocyte calmodulin. Equilibrium dialysis of *Xenopus* ovarian calmodulin against calculated free Ca concentrations, maintained with Ca-EGTA buffers, revealed a class of high-affinity binding sites. These sites had an apparent dissociation constant ( $K_d$ ) for Ca of  $2.8 \times 10^{-6}$  M. In our studies we detected two high-affinity calcium-binding sites per mole of protein (Fig. 1 and Table 1).

### Triggering of the $\text{G}_2\text{-M}$ Transition by the Calcium-Calmodulin Complex

Our previous studies have demonstrated that progesterone causes a rapid, but only transient increase in the free calcium activity in oocytes (32). Other studies have shown that the introduction of calcium into oocytes using ionophore A23187 or by iontophoresis, but not by pressure injections, can trigger the  $\text{G}_2\text{-M}$  transition in oocytes (17, 30). Perhaps, calmodulin is acting in the oocyte to mediate the effect of a rise in free calcium. Ionophore studies have shown that too high a calcium concentration is inhibitory to the resumption of meiosis (15, 30) and, therefore, calcium concentrations near the  $K_d$  of ovarian calmodulin were chosen. Calmodulin preincubated and injected in a Tris buffer that contained only 1  $\mu\text{M}$  calcium had no effect on the recipient oocytes. However, when the free Ca concentration was raised to approximately three times the measured  $K_d$  ( $2.8 \times 10^{-6}$  M), i.e., 10  $\mu\text{M}$ , positive results were obtained (Fig. 2). The resumption of meiosis in response to the calcium-calmodulin complex injections took place in the complete absence of any hormonal stimulation. There appeared to be an optimal amount of calcium-calmodulin complex that was effective in triggering the  $\text{G}_2\text{-M}$  transition. Between 60 and 80 ng, or  $\sim 3\text{--}4$  pmol, of calmodulin triggered the highest frequency of meiosis ( $\text{G}_2\text{-M}$  transition) (Fig. 2). The injection of free calcium alone had no effect on the recipient oocytes, thus confirming earlier results (Fig. 2). The magnesium (3 mM) in the injection buffer in combination with calmodulin could not induce oocyte maturation. Therefore, a specific amount of bound calcium and calmodulin was necessary to trigger the resumption of meiosis.

Calcium-calmodulin-induced meiosis followed the same time line as that normally induced by progesterone. Nuclear

TABLE I  
Physical Properties of *Xenopus* Ovarian Calmodulin

	Ovarian*	Other tissues (2, 8, 16)
mol wt		
Gel filtration	30,000	28,000–31,000
SDS PAGE	18,500	16,500–19,500
Isoelectric pH	4.3	3.9–4.3
$E_{276}^{1\%}$	2.2	2.0
Calcium binding (high affinity)		
mol Ca/mol protein	2	2–4
$K_d$	$2.8 \times 10^{-6}$ M	$1 \times 10^{-6}$ – $3 \times 10^{-6}$ M

\* A portion of this data has been published in a preliminary note (reference 27).

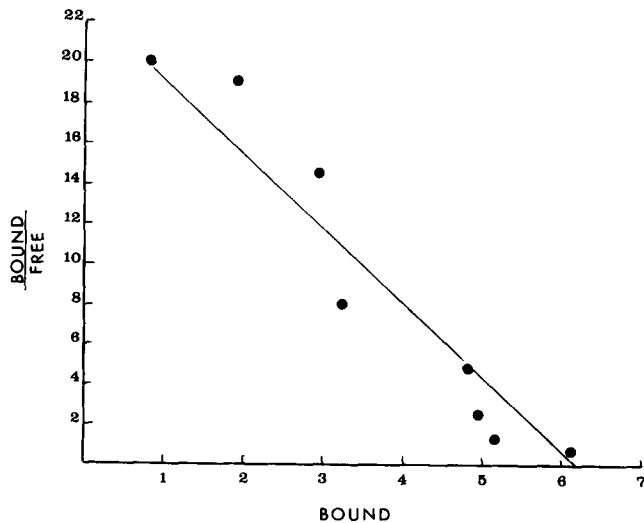


FIGURE 1 Scatchard plot of  $\text{Ca}^{2+}$  binding to oocyte calmodulin. Purified calmodulin was dissolved in 100 mM KCl, 10 mM imidazole, 0.1 mM EGTA, pH 7.0, at 500  $\mu\text{g}/\text{ml}$  and dialyzed overnight. 0.5-ml Samples were placed inside dialysis bags and dialyzed against 100 ml of buffer containing 10  $\mu\text{Ci}$  of  $^{45}\text{CaCl}_2$ . Desired free calcium concentrations, in equilibrium with EGTA, were obtained by adding known amounts of unlabeled  $\text{Ca}(\text{OH})_2$  according to the calculations of Rafflaub (21). The Ca-binding constant of EGTA has been corrected for pH 7.0. Dialysis was carried out for 48 h at 2°C with constant stirring. At the end of this period, duplicate 100- $\mu\text{l}$  aliquots from inside and outside of the dialysis bags were counted and the amount of calcium bound to calmodulin was calculated. Each aliquot from inside the bag contained 3 nmol of calmodulin. Bound: nmol calcium bound per 3 nmol calmodulin.

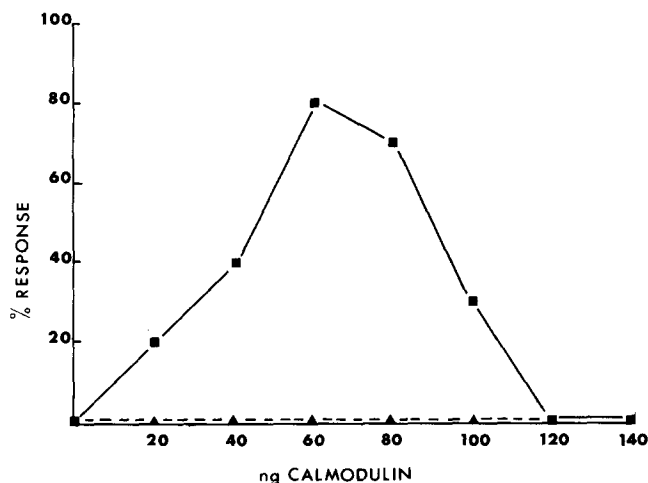


FIGURE 2 Calmodulin-induced oocyte maturation ( $\text{G}_2\text{-M}$  transition). Purified oocyte calmodulin was dissolved in 40 mM Tris-HCl, 3 mM  $\text{MgCl}_2$ , 0.01 mM  $\text{CaCl}_2$ , pH 8.0, at 2 mg/ml. This solution was microinjected into oocytes in volumes of 10, 20, 30, 40, 50, 60, and 70 nl delivering 20, 40, 60, 80, 100, 120 and 140 ng of calmodulin per oocyte, respectively. Each dose was injected into 20 recipient oocytes. Buffer containing calcium at 0.01 mM was injected by itself in the same 7 vol listed above. Oocytes were scored for nuclear membrane dissolution over a period of 7-8 h according to the procedure described in the text. ■, Samples containing calmodulin. ▲, Injection of buffer alone or calmodulin without calcium.

membrane dissolution was observed ~7 h after the injection of the complex. Thus, it appears that calmodulin is acting at a point in the sequence of events that is equivalent to the point of progesterone action. Therefore, we suggest that the increased

calcium activity after progesterone stimulation, coupled with calmodulin, somehow leads to the resumption of meiosis ( $\text{G}_2\text{-M}$  transition).

### Effect of Ovarian Calmodulin on Bovine Brain and Oocyte PDE Activity

One of the properties of authentic calmodulin is its ability to activate the soluble form of bovine brain PDE (calmodulin depleted) in a calcium-dependent manner (2, 8, 16). We compared the ability of bovine brain calmodulin and *Xenopus* calmodulin to activate bovine brain PDE. Both brain and *Xenopus* ovarian calmodulin stimulated mammalian brain PDE to the same extent and only in the presence of calcium; magnesium could not substitute for calcium (Fig. 3A). The ability of the protein, that we have isolated from *Xenopus* oocytes, to activate bovine brain PDE in a Ca-dependent manner, by definition, makes it a calcium-modulating protein. Various preparations of *Xenopus* calmodulin stimulated bovine brain PDE 3- to 10-fold in the presence of 0.1 mM calcium.

Calmodulin, isolated from *Xenopus* oocytes or from bovine brain, did not stimulate the soluble forms of cAMP or cGMP PDEs isolated from *Xenopus* oocytes (Fig. 3B). The calcium concentration was varied from 1  $\mu\text{M}$  to 1 mM, while the calmodulin concentration was varied from 10 ng to 10  $\mu\text{g}/\text{ml}$ . Therefore, calmodulin does not appear to play a functional role in regulating the activity of these PDEs in *Xenopus* oocytes. Both bovine brain and oocyte PDEs were passed through the calmodulin affinity column one at a time. As seen in Fig. 4,

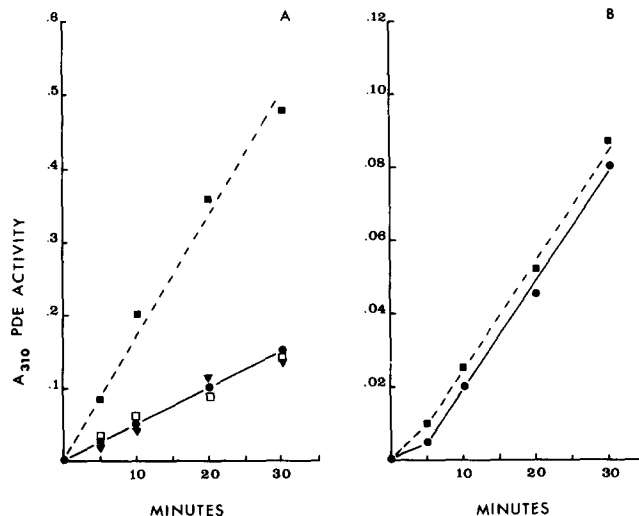


FIGURE 3 Calmodulin stimulation of bovine brain PDE, but not oocyte PDE. (A) The assay for the stimulation of bovine brain PDE (post DE-52) was performed in 40 mM Tris-HCl, 3 mM  $\text{MgCl}_2$ , pH 8.0, buffer. Brain PDE was added to the reaction buffer at a final concentration of 100  $\mu\text{g}/\text{ml}$ , while brain or ovarian calmodulin was added to a final 10  $\mu\text{g}/\text{ml}$ . The reaction was carried out at 30°C. PDE activity was measured by the method published by Watterson et al. (33). Bovine brain and ovarian calmodulin gave similar results. ▼, 0  $\mu\text{g}/\text{ml}$  Calmodulin, 0 mM  $\text{CaCl}_2$ , 100  $\mu\text{g}/\text{ml}$  PDE. ●, 10  $\mu\text{g}/\text{ml}$  Calmodulin, 0 mM  $\text{CaCl}_2$ , 100  $\mu\text{g}/\text{ml}$  PDE. □, 0  $\mu\text{g}/\text{ml}$  Calmodulin, 0.1 mM  $\text{CaCl}_2$ , 100  $\mu\text{g}/\text{ml}$  PDE. ■, 10  $\mu\text{g}/\text{ml}$  Calmodulin, 0.1 mM  $\text{CaCl}_2$ , 100  $\mu\text{g}/\text{ml}$  PDE. (B) Oocyte PDE (post DE-52) was added to a final concentration of 100  $\mu\text{g}/\text{ml}$  with or without brain or ovarian calmodulin at 10  $\mu\text{g}/\text{ml}$  in the presence or absence of 0.1 mM  $\text{CaCl}_2$ . ●, 0  $\mu\text{g}/\text{ml}$  calmodulin, 0 mM  $\text{CaCl}_2$ . ■, 10  $\mu\text{g}/\text{ml}$  calmodulin, 0.1 mM  $\text{CaCl}_2$ . Other concentrations of Ca and calmodulin as described in the text gave similar negative results.

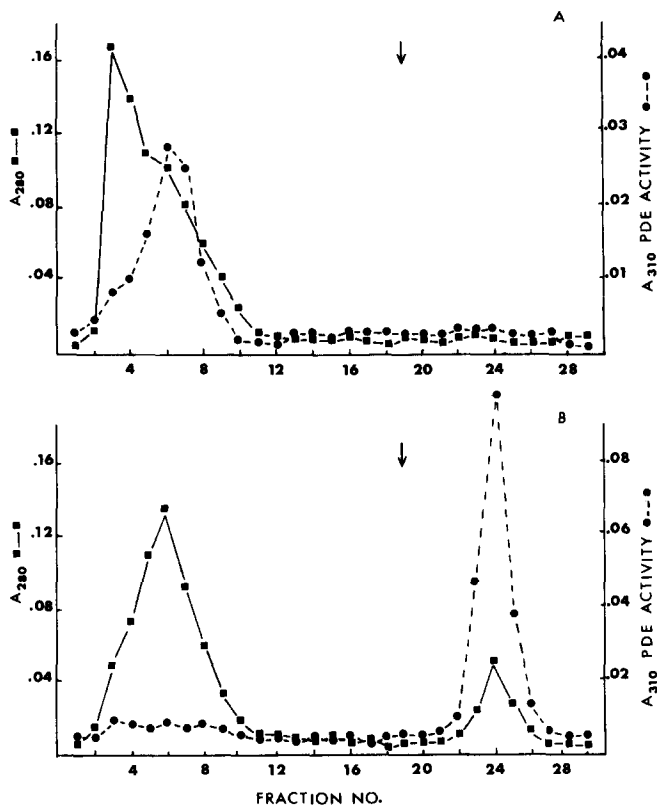


FIGURE 4 PDE binding to the calmodulin affinity column. (A) Approximately 10 mg of oocyte calmodulin was successfully coupled to CNBr-activated Sepharose 4B (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.) by the procedures of Klee and Krinks (7). Control oocyte PDE was dissolved in calcium-binding buffer which contained 40 mM Tris-HCl, pH 7.5, 50 mM NaCl, 0.2 mM  $\text{CaCl}_2$ , and 3 mM  $\text{MgCl}_2$ , and was dialyzed against this buffer overnight. 5 mg of this protein was passed through the affinity column in the calcium-binding buffer. After the unbound protein was washed off, the solution was changed to an EGTA-containing buffer (2 mM, arrow). Fractions were assayed for PDE activity as described in Fig. 3. (B) 5 mg of bovine brain PDE (post DE-52) in calcium-binding buffer was passed through the column as described above. The column was then challenged with the EGTA-containing elution buffer. Fractions were assayed for PDE activity as described in Fig. 3.

ovarian PDE, in a calcium-containing buffer, passed straight through the column in the void fraction (Fig. 4A). On the other hand, bovine brain PDE did bind to the column and was later eluted off with an EGTA-containing buffer (Fig. 4B). Thus, the binding characteristics of the two PDEs are consistent with their ability to be activated by calmodulin. A calcium-modulating protein has been isolated from a number of tissues that have the ability to activate bovine brain PDE in a Ca-dependent manner, yet these calmodulins are all unable to activate the PDE isolated from their respective tissue (3, 6, 28). Thus, the soluble forms of ovarian PDEs appear to fall into this latter category.

### Isolation of Calmodulin-regulated Proteins from *Xenopus* Oocytes

Ovarian homogenates were subjected to ammonium sulfate precipitation to produce four groups of proteins: those precipitating out between 0 and 30%, 30 and 55%, 55 and 65%, and 65 and 100% ammonium sulfate saturation. Each fraction was

passed through the column in a calcium-containing buffer. Only the proteins precipitating out between 30 and 55% ammonium sulfate saturation showed any significant binding to the calmodulin matrix. Approximately 10% of this protein fraction bound to the column and was later eluted off with the EGTA-containing buffer (Fig. 5A).

Several enzymes in addition to PDE are known to be regulated by calmodulin in other tissues (2, 8, 16). One of these is calcium-dependent protein kinase (24). Oocyte protein phosphorylation is known to increase twofold during the  $G_2$ -M transition in response to progesterone stimulation (10). Therefore, assaying for protein kinase activity in the fraction that bound to the affinity column seemed to be a logical first step. As seen in Fig. 5B, the bound fraction did contain a protein kinase activity that was dependent on the simultaneous presence of calcium and calmodulin in the assay reaction. The protein substrates that were phosphorylated in this assay con-

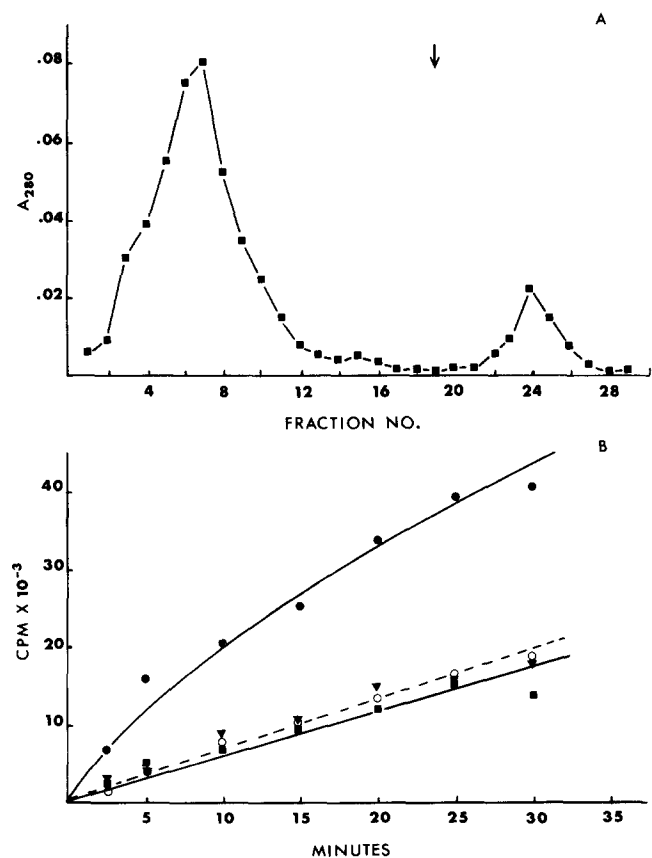


FIGURE 5 Oocyte protein binding to the calmodulin affinity column. (A) 5 mg of oocyte protein (30-50% ammonium sulfate cut) in calcium-binding buffer was passed through the column as described in Fig. 4. The column was then challenged with the EGTA-containing elution buffer (arrow). (B) The "bound" protein fraction eluting off the affinity column with EGTA was assayed for calcium-calmodulin-dependent protein kinase activity using the procedures of Schulman and Greengard (24). The proteins that went straight through the column ( $V_0$ ) in the presence of calcium were used as substrates for phosphorylation. The amount of [ $^{32}$ P]phosphate incorporated into protein from  $^{32}$ ATP was determined by the method of Maller et al. (10). The protein substrate concentration in all assays was 100  $\mu\text{g}/\text{ml}$ . ■, 0  $\mu\text{g}/\text{ml}$  bound protein, 0 mM  $\text{CaCl}_2$ , 10  $\mu\text{g}/\text{ml}$  calmodulin. ○, 0  $\mu\text{g}/\text{ml}$  bound protein, 1 mM  $\text{CaCl}_2$ , 10  $\mu\text{g}/\text{ml}$  calmodulin. ▼, 10  $\mu\text{g}/\text{ml}$  bound protein, 0 mM  $\text{CaCl}_2$ , 10  $\mu\text{g}/\text{ml}$  calmodulin. ●, 10  $\mu\text{g}/\text{ml}$  bound protein, 1 mM  $\text{CaCl}_2$ , 10  $\mu\text{g}/\text{ml}$  calmodulin.

sisted of the oocyte proteins that did not bind to the affinity column (void fraction, Fig. 5A). Thus, the oocyte contains at least one enzyme that is regulated by the calcium-calmodulin complex. Several other enzymes that are known to be regulated by calmodulin may also be present in this bound fraction off the affinity column. Further assays will have to be performed to confirm their absence or presence in this fraction.

## DISCUSSION

Amphibian oocytes, like most other cells or tissues examined, contain a calcium-binding protein that has most of the physical properties expected of authentic calmodulin. The calcium-binding properties of *Xenopus* ovarian calmodulin are slightly different than those reported by some workers using mammalian tissues. Dedman et al. (5) reported that rat testis calmodulin has four equivalent high-affinity calcium-binding sites having a  $K_d$  of  $2.4 \times 10^{-6}$  M. On the other hand, Watterson et al. (33) and Lin et al. (9) both reported that bovine brain calmodulin contains two sets of calcium-binding sites. One set consists of two high-affinity binding sites with a  $K_d$  of  $1-3 \times 10^{-6}$  M and two low-affinity sites having a  $K_d$  of  $2 \times 10^{-5}$  to  $8 \times 10^{-4}$  M. In our study, the free calcium concentration during equilibrium dialysis did not exceed  $1 \times 10^{-4}$  M. To measure low-affinity binding with a  $K_d$  of  $1 \times 10^{-4}$  M, we would have had to extend the free calcium concentration up to  $1 \times 10^{-2}$  M. Thus, any low-affinity calcium-binding sites, that may exist on *Xenopus* calmodulin, would not be seen in our data. Because a free calcium concentration of  $10^{-4}$  to  $10^{-3}$  M inside a cell does not exist, such low-affinity binding would probably have no physiological importance in terms of calmodulin function.

Our preliminary measurements indicate that each oocyte contains ~200 ng of calmodulin. Quite recently Cartaud et al. (4) have reported that the calmodulin content of a *Xenopus* oocyte increases from 110 to 170 ng during a 5-h period after progesterone stimulation. This would indicate a synthetic rate for calmodulin of 12 ng/h per oocyte. Measurements of the absolute rate of total protein synthesis in *Xenopus* oocytes show an increase from a control rate of 20 ng/h per oocyte to 40 ng/h per oocyte after progesterone-induced maturation.<sup>1</sup> Thus, the synthesis of calmodulin would have to represent over 40% of the total protein being synthesized during oocyte maturation. However, this high level of calmodulin synthesis is never seen on fluorographs of two-dimensional gels.<sup>1</sup> In fact, the level of calmodulin synthesis appears to be negligible in maturing oocytes. Perhaps, the apparent increase in calmodulin levels reported by Cartaud et al. (4) is caused by differential recoveries of calmodulin from control and maturing oocyte cytoplasts.

The full-grown amphibian oocyte represents a cell arrested in late prophase of the meiotic cell cycle. Thus, the resumption of meiosis (oocyte maturation) represents a cell undergoing a  $G_2$ -M transition. Calcium ions have been shown to play a key role in regulating this transition during the mitotic cell cycle (22, 27). Furthermore, Welsh et al. (34) have demonstrated that calmodulin undergoes a redistribution within a cell during the mitotic period. Thus, the potential exists for calcium and calmodulin to play a key role in regulating some events of the  $G_2$ -M transition. Our study clearly demonstrates that calmodulin, in the form of a calcium-calmodulin complex, can regulate the initiation of the  $G_2$ -M transition in oocytes. The complex appears to be able to completely replace the normal trigger for the resumption of meiosis, i.e., progesterone. Similar

results have recently been reported by Maller and Krebs (13). A similarly shaped dose-response curve has been reported for Ca-ionophore A23187-induced oocyte maturation (30). It appears that too high a  $Ca^{2+}$  concentration inhibits oocyte maturation. Therefore, the transient increase in free calcium that normally takes place in response to progesterone stimulation could well be mediated by calmodulin within the oocyte.

Conflicting evidence exists in the literature concerning the role of cAMP in controlling the resumption of meiosis in the amphibian oocyte. Some studies show no change in the cAMP content of oocytes during the  $G_2$ -M transition (19, review 31). On the contrary, other studies demonstrated a 50–60% decline in the cAMP pool shortly after progesterone stimulation (12, 18, 25). One mechanism that could account for this potential drop in cAMP would be an increased degradation of cAMP by oocyte PDE (11, 12). Certain forms of PDE in other tissues are known to be regulated by calmodulin (2, 8, 16). Ovarian calmodulin has the ability to stimulate the soluble form of bovine brain cAMP and cGMP PDEs in a calcium-dependent manner. On the contrary, calmodulin does not have the ability to stimulate the soluble form of ovarian cAMP and cGMP PDEs. This result does not preclude the possibility that a particulate form of ovarian PDE is under the control of the calcium-calmodulin complex. However, at present, we are unable to demonstrate the existence of a particulate form of ovarian PDE that is activated by calmodulin. Therefore, the possible involvement of a drop in cAMP brought about by a calcium-calmodulin dependent PDE, in controlling the  $G_2$ -M transition, cannot be substantiated at this time.

Calmodulin is now known to regulate several enzymes in other tissues in a calcium-dependent manner. These include PDE, adenylate cyclase, Ca-ATPase, myosin light chain kinase, nicotinamide adenine dinucleotide kinase, phosphorylase kinase, and protein kinase. In addition, the process of microtubule depolymerization also appears to be regulated by the calcium-calmodulin complex (2, 8, 16). One potential role for calmodulin in *Xenopus* oocytes appears to be at the level of protein kinase regulation. Our study shows that oocytes contain a cAMP-independent protein kinase that is dependent on the simultaneous presence of calcium and calmodulin for its maximal activity. As mentioned earlier, protein phosphorylation increases approximately two- to threefold before nuclear membrane dissolution in *Xenopus* oocytes (10). Furthermore, there does not appear to be a change in the specific activity of cAMP-dependent protein kinases during the  $G_2$ -M transition (20). Therefore, the increased protein phosphorylation may be the result of an increase in calcium-calmodulin-dependent protein kinase activity. The nature and function of the proteins that are phosphorylated by this kinase(s) have not been determined as yet.

Further work will be needed to catalogue all the oocyte proteins that are regulated by the calcium-calmodulin complex. Once this is accomplished, then it must be determined how these proteins may be involved in triggering the resumption of meiosis in prophase-arrested oocytes.

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