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Changes in the Levels of Calmodulin and of a Calmodulin Inhibitor in the Early Phases of Radish (*Raphanus sativus* L.) Seed Germination

EFFECTS OF ABA AND FUSICOCCIN[1]

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ABSTRACT

An inhibitor of Ca^{2+}-calmodulin (Cam)-dependent brain phosphodiesterase was present in the soluble fraction of embryo axes from ungerminated radish (*Raphanus sativus* L.) seeds. This inhibitor is a Ca^{2+}-dependent, Cam-binding protein; in fact: (a) its effect was strongly reduced by treatment with proteases; (b) the inhibition was counteracted by Cam but not by Ca^{2+}; (c) on gel filtration in the presence of Ca^{2+}, Cam co-chromatographed with the inhibitor. The inhibitor is heat stable and positively charged at pH 7.5. During early phases of germination, the fresh weight and the levels of DNA and RNA of embryo axes increased, the level of the inhibitor decreased, and the level of Cam increased. Abscisic acid (ABA) inhibited germination, the decrease of inhibitor, and the increase of Cam. Fusicoccin (FC) stimulated the increase in fresh weight but not the increase in the RNA and DNA levels; in this condition, the inhibitor level decreased and the increase in Cam level was higher than in the control. In the presence of both ABA and FC, there was an increase in fresh weight not accompanied by an increase in DNA and RNA levels; Cam increased and, on a fresh weight basis, reached the value of the control. These results indicate that the Ca^{2+}-Cam system was activated in early germination of radish seeds by an increase in Cam and a decrease in the inhibitor levels, that FC, probably through the activation of membrane functions, increased Cam level, and that the ABA inhibition on germination was not mediated by the Ca^{2+}-Cam system.

Calcium and Cam[2] are involved in many physiological processes in animal and plant cells. In plants the effects of Cam antagonists such as chlorpromazine, fluphenazine, and trifluoperazine suggest a role of the Ca^{2+}-Cam system in the responses to auxin, cytokinin, and gibberellic acid (11, 24), in phytochrome responses (26), in protoplast fusion (13), in volume regulation (16), and in polar growth of tips and pollen tubes (14). The enzymes so far known to be dependent on Ca^{2+}-Cam are: Ca^{2+}-ATPase (10), NAD^+ kinase (1), and kinases of soluble and membrane-bound proteins (20, 22, 23, 30). These activities can be regulated by the Ca^{2+}-Cam system either through a change in the Ca^{2+} concentration in the cytosol, which can act as a second cellular messenger, or by the Cam level. Studying cell growth and functional differentiation in animal systems, Cam levels appear to be related to the proportion of cells in dividing and nondividing state (28), to the fertilization of sea urchin eggs (12), to the maturation of oocytes of *Xenopus* (6), and to DNA synthesis and mitosis (27). In plants a relationship between Cam level and cell growth is suggested by the fact that higher levels (17-fold) of Cam were found in the root apex (meristem) with respect to the zone of cell elongation (1) and by results on seed germination. Cam level increases during early phases of germination; this increase is reduced when germination is inhibited by ABA, and Cam level appears to be a limiting factor for the Ca^{2+}-Cam-dependent activities (8).

An inhibitor of Ca^{2+}-Cam-dependent PDE is present in radish (*Raphanus sativus* L.) seeds. This inhibitor could be involved in the regulation of Ca^{2+}-Cam-dependent activities and, consequently, of seed germination (8).

Seed germination is also characterized by the reactivation of membrane functions (4, 9). FC, a toxin able to stimulate proton extrusion and K^+ absorption and to increase the negative electric transmembrane potential (19), stimulates germination, while ABA inhibits germination and the reactivation of membrane functions. FC reverses, in early germination phases, the inhibition of ABA on membrane functions and germination (4).

The present paper deals with the evidence that the inhibitor, present in radish seeds, is a Cam-binding protein and reports the changes in Cam and inhibitor levels during early germination phases of radish embryos when germination was stimulated by FC or inhibited by ABA. The role of Cam and of the inhibitor in germination is discussed in relation to membrane reactivation and ABA inhibiting effect.

MATERIALS AND METHODS

Plant Material and Incubation of Conditions. Radish seeds (*R. sativus* L., cv Tondo Rosso Quarantino, obtained from Ingegnoli, Milan, Italy) from a single year's crop were used. Seeds were decoated and incubated as described in a previous paper (9). At the desired incubation times, the embryonal axes were excised and weighed as previously described (4).

Chemicals. Fluphenazine·2HCl was kindly provided by E. R. Squibb and Sons, Inc., Princeton, NJ; FC was a gift from Farmoplant s.p.a., Italy; cAMP, adenosine deaminase, alkaline phosphatase, leucine aminopeptidase, and papain were purchased from Boehringer Biochemia Robin, Italy; Tris, Hepes, Mes, ABA, phenylmethylsulfonyl fluoride, BSA, and Cyt *c* were from Sigma; PAGE reagents, SDS-PAGE low M_r standards were from Bio-Rad Laboratories; Sephadex and Sepharose 6B, epoxy activated, were purchased from Pharmacia Fine Chemicals, Swe-

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[2] Abbreviations: Cam, radish calmodulin; FC, fusicoccin; PDE, brain phosphodiesterase.

den; CM- and DEAE-cellulose were from Whatman, England. Bolton and Hunter reagent was from Amersham, England.

Preparation of Cell Fractions. Radish embryos were homogenized with a mortar in 5 volumes of a medium containing 50 mM Hepes, 70 mM KCl, 10 mM $MgCl^2$, 250 mM sucrose, pH 7.2; immediately before use 15 mM β-mercaptoethanol and 30 μg/mL of phenylmethylsulfonyl fluoride (as solution in DMSO, 15 mg/mL) were added. The homogenate was strained through a layer of muslin and centrifuged at 500*g* for 10 min, at 13,000*g* for 15 min, and at 100,000*g* for 60 min at 2°C. The postmicrosomal supernatant was analyzed on a Sephadex G-75 SF column (1.4 × 60 cm), and the fractions were tested for Cam, inhibitor, and protein content. Alternatively, Cam levels were assayed in the 100,000*g* supernatant desalted by gel filtration on a Sephadex G-25 column and freed from the inhibitor by a passage through a CM-cellulose column conditioned with 10 mM Hepes, pH 7.5 (the inhibitor is positively charged at pH 7.5 and is bound to cation exchangers). Inhibitor levels were assayed in the 100,000*g* supernatant desalted by a gel filtration on a Sephadex G-25 column and freed from Cam by a passage through a DEAE-cellulose column conditioned with 10 mM Hepes, pH 7.5 (Cam is negatively charged at pH 7.5 and is bound to anion exchangers).

Extraction and Purification of Cam. Radish seeds were extracted according to Kakiuchi *et al.* (15). Cam was purified by chromatography on DEAE-cellulose (pH 7.5) eluted with 0.5 M $(NH_4)_2SO_4$, followed by fluphenazine-Sepharose 6B column affinity chromatography and elution with EGTA, essentially as described for plant Cam (7). The Cam purified as above was homogeneous when analyzed by electrophoresis on 12% polyacrylamide gels in the presence of SDS (17).

Cam was labeled with ^{125}I using the Bolton and Hunter reagent.

Assay of Cam Level. Cam was assayed by its capacity to activate PDE. Cam-free PDE was prepared according to Yamagami and Terayama (29). PDE was spectrophotometrically assayed in 1 mL final volume, in the presence of alkaline phosphatase or 5′-nucleotidase and adenosine deaminase, in a Beckman DU 50 spectrophotometer. Cam content was calculated by comparing the activation produced by the fractions with that observed in the presence of purified Cam (8).

Assay of the Inhibitor Level. The inhibitor was assayed by its capacity to inhibit PDE activated by an amount of Cam which did not saturate PDE. PDE activity in the presence of the amount of Cam (0.55 μg) needed to give 70% maximum activation was taken as reference value (100%); one unit of inhibitor was defined as the amount which reduced this activity to 50%.

PAGE. Nondenaturing homogeneous gel and buffer system 15% PAGE was performed using 50 mM Tris-Cl buffer, pH 7.5 (3). Nondenaturing, multiphasic, low pH, discontinuous 15% PAGE was performed as described by Andrews (3). SDS-PAGE in the presence of urea was performed using the discontinuous system described by Anderson *et al.* (2).

DNA, RNA, and Protein Levels. DNA, RNA, and protein contents in germinating radish seeds were assayed according to Nieman and Poulsen (21). Protein concentration was determined by the methods of both Lowry *et al.* (18) and Bradford (5), using BSA and γ-globulin, respectively, as standards.

RESULTS

Identification and Characterization of an Inhibitor of Cam in the Soluble Fraction of Radish Embryos. Figure 1 shows the elution pattern, in the presence of Ca^{2+}, of the soluble fraction (100,000*g*) of embryo axes from ungerminated radish seeds. Cam was eluted in a main peak in the zone corresponding to M_r 36,500 D; a low amount of Cam was found at higher M_r.

The fractions eluted in the zone at low M_r (about 13,000 D) contained an inhibitor of Ca^{2+}-Cam-activated PDE. These fractions (from 28 to 32) were pooled and used as source of the inhibitor.

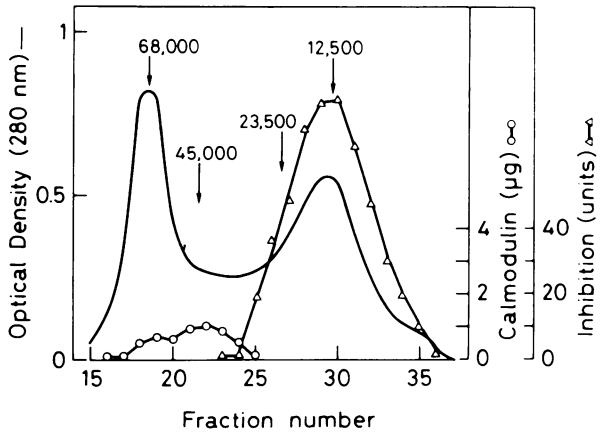

FIG. 1. Separation of the soluble fraction of embryo axes of radish seeds at 0 h of germination on a Sephadex G-75 SF column. The soluble fraction (0.9 ml corresponding to about 120 embryos) was applied to the column (1.4 × 60 cm). The elution buffer contained: 10 mM Hepes, 50 mM KCl, 0.5 mM $CaCl^2$ (pH 7.5). Protein concentration was assayed by measuring A_{280} (——). Cam contents were measured (activation of PDE) in the fractions (1.75 mL) denatured by heating and freed from the inhibitor by chromatography on CM-cellulose (O——O). Inhibitor contents were measured in the fractions denatured by heating and freed from Cam by chromatography on DEAE-cellulose (Δ——Δ). The arrows indicate M_r values of standard proteins. Low M_r molecules (dinitrophenol was used as standard) were eluted at fraction 46.

Heat treatment (90°C for 5 min) of the inhibitor did not affect its activity on the Cam-activated PDE (data not shown).

Treatments with either leucine aminopeptidase or papain (incubation at 37°C in the presence of 10 units/mL) reduced the activity of the inhibitor to less than 10% of the control (data not shown).

The inhibitor was eluted in a single symmetrical peak in the zone at about M_r 13,000 D when analyzed on a Sephadex G-50 SF column (1.4 × 60 cm) (data not shown). Ion exchange chromatography at pH 7.5 shows that the inhibitor was not bound to anion exchanger (DEAE-cellulose) but it is bound to cation exchanger (CM-cellulose) and was eluted at 0.3 M KCl concentration. In agreement with these results, the inhibitor on nondenaturing 15% PAGE at pH 7.5 migrated toward the cathode in a single band (Fig. 2). On nondenaturing discontinuous 15% PAGE system at low pH (separating gel at pH 3.8), the inhibitor was resolved in two bands. When the inhibitor was analyzed on SDS-PAGE in the presence of 8 M urea, it was separated in two bands showing about M_r 9,500 D ± 7% (SD) and about M_r 5,000 D ± 5% (SD) (Fig. 2).

Figure 3 shows that increasing concentrations of the inhibitor progressively inhibited Cam-activated PDE; the amount of inhibitor required to inhibit Cam-activated PDE was higher when the PDE was assayed in the presence of higher amounts of Cam. An increase of calcium in the test assay mixture of PDE from 0.2 to 10 mM did not affect the inhibitor activity (data not shown).

Figure 4A shows that the elution peak of Cam on Sephadex G-75 SF column chromatography shifted from 23,500 D (characteristic of Cam on gel filtration), progressively with increasing amounts of the inhibitor, to zone of higher M_r in the presence of higher amounts of the inhibitor, Cam was eluted in the zone corresponding to about M_r 36,500 D. When the elution was performed in the absence of Ca^{2+} (0.5 mM EGTA) (Fig. 4B), Cam elution was not modified by the presence of the inhibitor.

Growth of Radish Seed Embryo Axes. Radish embryo axes increased in length and in fresh weight during early phases of

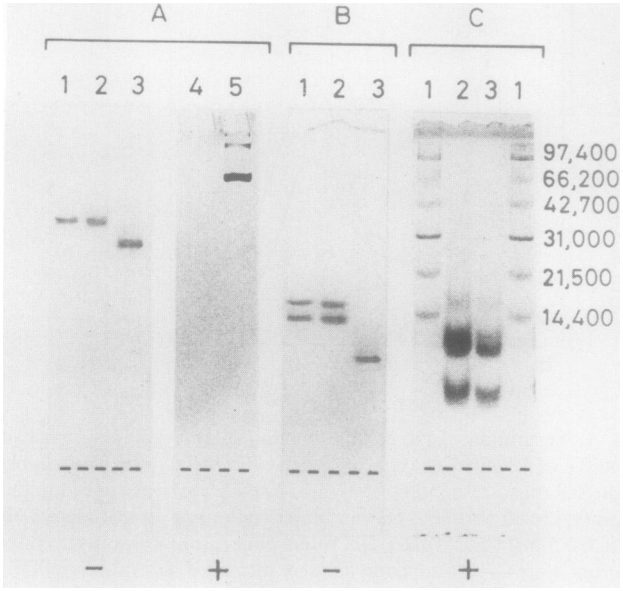

FIG. 2. PAGE of the inhibitor. Coomassie brilliant blue stain. Dye position is indicated by the dashed lines. A, Homogeneous nondenaturing PAGE at pH 7.5, migration toward the cathode: lane 1, 5 μL inhibitor (1 mg/mL protein); lane 2, 10 μL inhibitor (1 mg/mL protein); lane 3, 10 μL Cyt *c* (1 mg/mL protein); migration toward the anode: lane 4, 10 μL (1 mg/mL protein); lane 5, 10 μL BSA (1 mg/mL protein). B, Discontinuous nondenaturing PAGE at pH 3.8, migration toward the cathode: lane 1, 5 μL inhibitor (1 mg/mL protein); lane 2, 10 μL inhibitor (1 mg/mL protein); lane 3, 10 μL Cyt *c* (1 mg/mL protein). C, SDS-PAGE in the presence of urea: lane 1 and 4 protein standards; lane 2, 40 μL inhibitor (0.25 mg/mL protein); lane 3, 20 μL inhibitor (0.25 mg/mL protein). The electrophoretic runs were repeated three times.

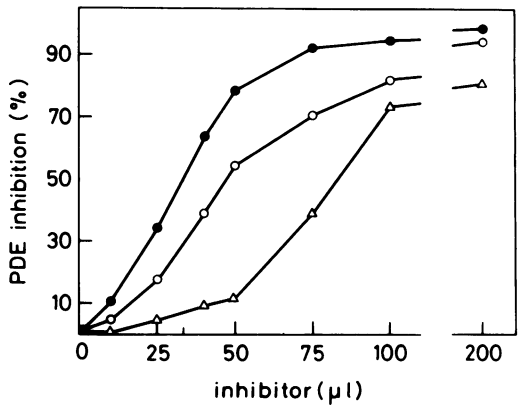

FIG. 3. Effects of the inhibitor on PDE activated by several concentrations of Cam. PDE assay was performed in standard conditions in the presence of 0.55 μg Cam (●——●), the amount needed to give 70% maximum PDE activation; 1.1 μg Cam (○——○), the amount giving 90% maximum PDE activation; and 2.2 μg Cam (△——△), the amount oversaturating PDE. One unit of inhibitor is the amount reducing 70% Cam-activated PDE to 50%.

germination. Table I shows that fresh weight of the embryo axes increased by 166% during the first 24 h of incubation in water. ABA strongly inhibited (95%) this increase, while FC produced a further 50% increase with respect to the control. The inhibiting effect of ABA was reduced by FC.

The levels of RNA and DNA increased by about 120 and 70%, respectively, during the first 24 h of radish seed germination. The increase in RNA and DNA was very similar for the seeds

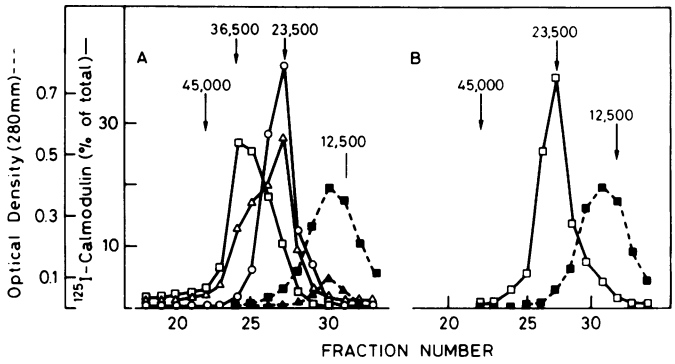

FIG. 4. Chromatography of ^{125}I-labeled radish Cam on a Sephadex G-75 SF column in the presence of the inhibitor of Cam-activated PDE. Column characteristics as specified in Figure 1. A, Column conditioned with 10 mM Hepes, 50 mM KCl, 0.5 mM $CaCl^2$ (pH 7.5): elution pattern of 0.5 μg of ^{125}I-Cam alone (○——○), of 0.5 μg of ^{125}I-Cam (△——△) in the presence of 0.5 A_{280} of the inhibitor (▲--▲); and of 0.5 μg of ^{125}I-Cam (□——□) in the presence of 2 A_{280} of the inhibitor (■--■). B, Column conditioned with the same buffer containing 0.5 mM EGTA instead of $CaCl^2$: elution pattern of 0.5 μg of ^{125}I-Cam (□——□) in the presence of 2 A_{280} of the inhibitor (■--■). The arrows indicate the M_r of standard proteins.

Table I. *Increase in Fresh Weight and DNA and RNA Levels of Embryo Axes from Germinating Radish Seeds*

Decoated radish seeds were incubated at 26°C in an agitated water bath; at the indicated times the embryo axes were excised and fresh weight, DNA and RNA were determined. The data are the mean values of six experiments. SD did not exceed ±13% for fresh weight, ±11% for DNA, and ±9% for RNA levels.

Incubation Conditions (Time and Treatment)	Fresh Wt	DNA	RNA
	g × *(100 emb.)*$^{-1}$	*μg* × *(100 emb.)*$^{-1}$	*mg* × *(100 emb.)*$^{-1}$
0 h	0.208	28	1.1
24 h, H_2O	0.554 (166)[a]	48 (71)	2.4 (118)
24 h, 8×10^{-5} M ABA	0.225 (8)	37 (32)	1.7 (55)
24 h, 10^{-5} M FC	0.720 (246)	46 (64)	2.3 (109)
24 h, 8×10^{-5} M ABA + 10^{-5} M FC	0.287 (38)	34 (21)	1.7 (55)

[a] Values in parentheses indicate the percentage of increase with respect to 0 time.

incubated in FC with respect to the seeds in water and for the seeds incubated in ABA+FC with respect to the seeds in ABA (data not shown).

Calmodulin and Calmodulin Inhibitor Levels. Table II reports the Cam and the Cam inhibitor levels present in the soluble fraction of radish embryo axes on the basis of both embryo number and fresh weight at 24 h of incubation. The Cam level, on an embryo number basis, increased by about 450% during the first 24 h of incubation in water. ABA strongly inhibited this increase, and at 24 h the amount of Cam was only 25% higher than that of ungerminated embryos. Cam increase was greatly stimulated by FC (750% with respect to the ungerminated embryos) and Cam level at 24 h was 54% higher than in the control. In the ABA+FC-treated seeds, Cam increased by about 170% with respect to the ungerminated embryos, and the increase of Cam was about 590% higher with respect to that of the ABA-treated seeds. The increase in Cam during the first 24 h of germination in water was still large, even expressing Cam level on a fresh weight basis (Cam content). The Cam content of seeds incubated in ABA was 15% higher with respect to that of

ungerminated seeds and 45% lower with respect to the control in water. The FC-treated seeds showed a Cam content 19% higher with respect to the control in water. The Cam content of the seeds incubated in ABA+FC reached a value very similar to that of seeds germinated in water.

Table II shows also that the levels of the inhibitor at 24 h of incubation in water or in FC were less than 6% of that present in the ungerminated seeds. ABA strongly inhibited the decrease of Cam inhibitor.

Table II. *Cam and Inhibitor Levels in the Soluble Fraction of Embryos from Germinating Radish Seeds*

The soluble fractions (100,000*g*) of radish embryos were passed through a DEAE-cellulose column to assay Cam and through a CM-cellulose column to assay the inhibitor; after heat denaturation, Cam content was assayed by the activation of PDE and inhibitor was assayed by the inhibition of Cam-activated PDE. The data are the mean values of three experiments. SD did not exceed ±11% for the Cam levels and ± 15% for the inhibitor levels.

Incubation Conditions (Time and Treatment)	Cam		Inhibitor	
	$\mu g \times$ *(100 emb.)*$^{-1}$	$\mu g \times$ *(g fr wt)*$^{-1}$	$u^a \times$ *(100 emb.)*$^{-1}$	$u \times$ *(g fr wt)*$^{-1}$
0 h	5.3	25.5	510	2455
24 h, H_2O	29.2	52.7	31	57
24 h, 8×10^{-5} M ABA	6.6	29.3	445	1978
24 h, 10^{-5} M FC	45.1	62.6	26	37
24 h, 8×10^{-5} M ABA + 10^{-5} M FC	14.4	50.2	353	1232

[a] u: one inhibitor unit corresponds to the amount which reduces to 50% the activity of the PDE, assayed in standard conditions, in the presence of the amount of Cam needed to give 70% maximum PDE activation.

Separation on Sephadex G-75 SF Column of Cam and Cam Inhibitor Present in the Soluble Fraction. Figure 5 shows that the peak of protein eluted in the low M_r zone decreased (compare with Fig. 1) during the first 24 h of incubation in water or FC. This decrease did not take place in seeds incubated in the presence of ABA- and ABA+FC-treated seeds.

Cam in seeds incubated 24 h in water was eluted in some shallow peaks in the zone corresponding to an M_r higher than about 30,000 D (fractions up to 25). When the elution was performed in the absence of Ca^{2+}, less than 10% of total Cam was eluted in the zone at M_r higher than 30,000 D (data not shown).

The Cam present in the zone of M_r lower than 30,000 D can be considered as "free" Cam (*i.e.* not bound to other structures). This fraction accounted for 7 and 5% of total Cam present in the soluble fraction for the seeds incubated in water or in FC, respectively. Cam from seeds incubated in ABA+FC was eluted in a very large zone comprehending M_r lower than 30,000 D: "free" Cam, in fact, was calculated to be more than 22% of total Cam.

The inhibitor was eluted in the zone of M_r of about 13,000 D, and its level was low for the seeds incubated in water or FC and high for the seeds incubated in ABA or ABA+FC.

DISCUSSION

An inhibitor of Ca^{2+}-Cam-activated PDE was present in the soluble fraction of radish embryo axes from ungerminated radish seeds. The fact that its activity was abolished by treatment with proteases strongly suggests that the inhibitor is a protein. The inhibitor is heat stable (5 min at 90°C), positively charged at pH 7.5; it is resolved in two bands on SDS-PAGE in the presence of urea showing M_r 9500 D ± 7% (SD) and M_r 5000 D ± 5% (SD), but it is eluted in a single peak on gel filtration (Sephadex G-50

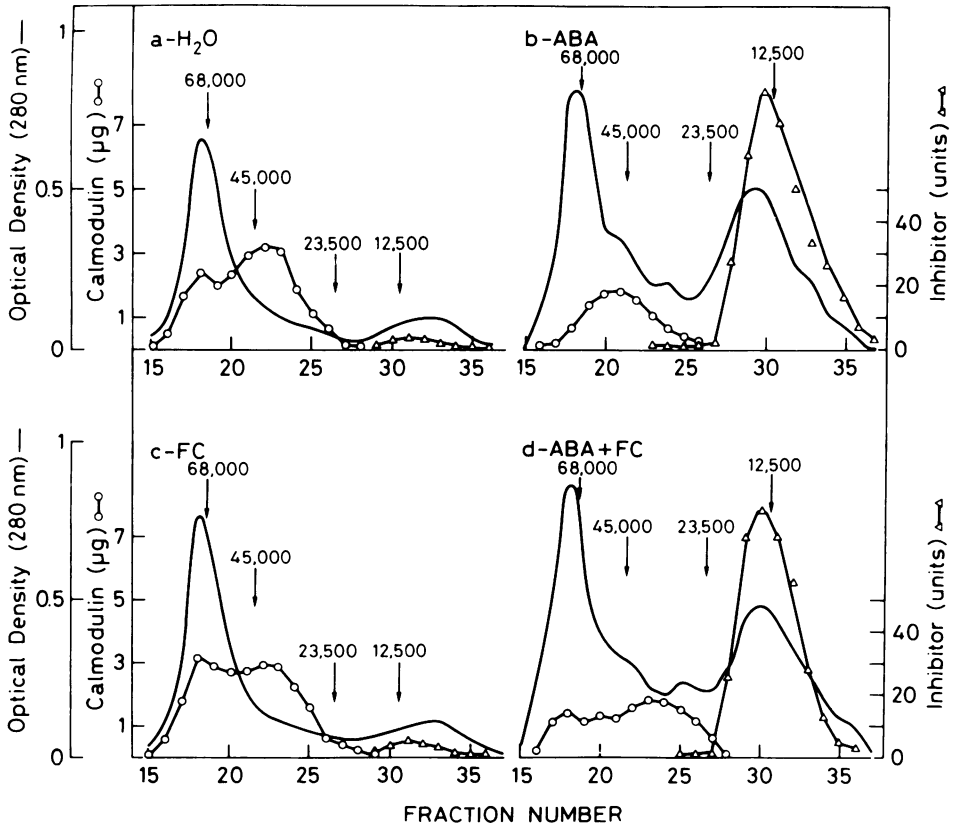

FIG. 5. Distribution of Cam and of the inhibitor present in the soluble fraction of embryos from radish seeds incubated for 24 h in (a) water, (b) ABA, (c) FC, and (d) ABA+FC on a Sephadex G-75 SF column in the present of Ca^{2+}. Decoated radish seeds were incubated at 26°C in an agitated water bath in the presence of H_2O, 8×10^{-5} M ABA, 10^{-5} M FC, and 8×10^{-5} M ABA + 10^{-5} M FC; after 24 h of incubation the embryos were excised and the soluble fraction was obtained and chromatographed on a Sephadex G-75 SF column. The amount of soluble fraction applied to the column corresponded to 72 embryos for the seeds in water, 94 embryos for the seeds in ABA, 60 embryos for the seeds in FC, and 94 embryos for the seeds in ABA+FC. The condition of chromatography, the determination of protein, and the assay tests of Cam and of the inhibitor are specified in Figure 1. Proteins, A_{280} (——); Cam, μg (○——○); and inhibitor, units (△——△).

SF) and on ion exchange chromatography (CM-cellulose) and migrated as a single band on nondenaturing PAGE at neutral pH. The role of the single protein bands resolved on urea SDS-PAGE in PDE inhibition is not clarified; the fact that the inhibitor, in nondenaturing conditions, appears as a single protein indicates that the two bands co-purify and might suggest the involvement of both in PDE inhibition. The effect of the inhibitor on PDE was counteracted by higher concentrations of Cam, and, since the inhibition was not reduced by higher Ca^{2+} concentrations, the inhibitor is not a Ca^{2+}-sequestering protein. On Sephadex G-75 SF in the presence of Ca^{2+}, Cam co-chromatographed with the inhibitor, and in the absence of Ca^{2+}, Cam and inhibitor were separated according to their M_r. These results indicate that PDE inhibition depends on the competition of the inhibitor for Ca^{2+}-Cam complex and that the inhibitor is a Ca^{2+}-dependent, Cam-binding protein.

At the moment no biochemical function has been identified for the inhibitor, and consequently its action appears to be linked to its capacity to compete for Ca^{2+}-Cam complex with the Ca^{2+}-Cam-dependent activities.

The strong decrease in the inhibitor level during early germination either in water or in the presence of FC and the lack of this decrease when germination was inhibited by ABA suggest that germination, with a complete metabolic seed reactivation, takes place only when the level of the inhibitor is low. The inhibitor, competing for Ca^{2+}-Cam complex with the Ca^{2+}-Cam-dependent activities, can play a regulatory role in early germination phases.

The strong increase in Cam level during the early 24 h of germination (Table II), the almost complete inhibition of this increase when germination was inhibited by ABA, and the stimulation of the Cam increase when germination was promoted by FC suggest a relationship between Cam level and the reactivation of seed growth and metabolism. The very low level of "free" (not bound to other structures) Cam in seeds germinated in water or in FC (less than 7% of total Cam present in the soluble fraction) indicates that Cam level is lower with respect to the present Ca^{2+}-Cam-dependent activities and suggests that Cam is a limiting factor in early radish seed germination.

FC promotes germination both in the control and in ABA-treated seeds and activates membrane functions (4, 9, 19); this activation is not accompanied by an increase in the RNA and DNA levels. These results suggest that the effect of FC is due to cell enlargement and not to a general metabolic activation. The fact that FC increased Cam levels in the control and in ABA-treated seeds suggests that FC or, more probably, the activation of membrane functions (directly or indirectly through some related metabolic process) stimulates the Ca^{2+}-Cam system by increasing the Cam level.

The high level of Cam (very similar to the controls) in the ABA+FC-treated seeds suggests that the inhibiting effect of ABA on germination is not mediated by an inhibition of the Ca^{2+}-Cam system; the higher amount of Cam present in the soluble fraction in "free" form (22% compared to less than 7% for the control) allows us to hypothesize that ABA inhibits germination by controlling the development of some activity dependent on the Ca^{2+}-Cam system.

In conclusion, during early germination of radish seeds, the precocious activation of plasma membrane could stimulate the Ca^{2+}-Cam system through an increase in Cam level. The activation of the Ca^{2+}-Cam system depends also on the lowering of the level of the inhibitor.

The Ca^{2+}-Cam system can be involved in seed germination through the control of some Ca^{2+}-Cam-dependent activity. NAD^+ kinase, a Ca^{2+}-Cam-dependent enzyme involved in the activation of sea urchin eggs (12), could also play a role in seed germination. NAD^+ kinase could increase the $NADP^+$ level and consequently promote the reactivation of the pentose phosphate pathway, which takes place in early germination phases (25). A role of Cam can be also hypothesized in the control of DNA synthesis and cell mitosis (27).

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