Correlation between Calmodulin Activity and Gravitropic Sensitivity in Primary Roots of Maize

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ABSTRACT

Recent evidence indicates a role for calcium and calmodulin in the gravitropic response of primary roots of maize (Zea mays L.). We examined this possibility by testing the relationship between calmodulin activity and gravitropic sensitivity in roots of the maize cultivars Merit and B73 × Missouri 17. Roots of the Merit cultivar require light to be gravitropically competent. The gravitropic response of the Missouri cultivar is independent of light. The occurrence of calmodulin in primary roots of these maize cultivars was tested by affinity gel chromatography followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis with bovine brain calmodulin as standard. The distribution of calmodulin activity was measured using both the phosphodiesterase and NAD kinase assays for calmodulin. These assays were performed on whole tissue segments, crude extracts, and purified extracts. In light-grown seedlings of the Merit cultivar or in either dark- or light-grown seedlings of the Missouri cultivar, calmodulin activity per millimeter of root tissue was about 4-fold higher in the apical millimeter than in the subapical 3 millimeters. Calmodulin activity was very low in the apical millimeter of roots of dark-grown (gravitropically nonresponsive) seedlings of the Merit cultivar. Upon illumination, the calmodulin activity in the apical millimeter increased to a level comparable to that of light-grown seedlings and the roots became gravitropically competent. The time course of the development of gravitropic sensitivity following illumination paralleled the time course of the increase in calmodulin activity in the apical millimeter of the root. The results are consistent with the suggestion that calmodulin plays an important role in the gravitropic response of roots.

Evidence in support of a role for calcium redistribution in gravitropism is provided by experiments showing that: (a) calcium chelating agents such as EGTA2 can prevent gravitropism in roots (14) and shoots (7), and (b) asymmetric application of calcium to the tips of vertically oriented primary roots of maize induces curvature toward the calcium (14).

The evidence that calcium plays a regulatory role in gravitropism raises the question of whether or not the action of calcium might be mediated by CaM. Calmodulin has been identified in both roots (1, 6, 11) and coleoptiles (3, 4) and there is limited evidence implicating CaM in the gravitropic mechanism. Immunocytochemical studies of CaM distribution in root caps of pea seedlings (6) show intense staining in the columella region, the region where the perception of gravity is thought to occur. Exposure of Avena seedlings to the CaM inhibitor, CPZ, inhibits coleoptile gravitropism (2) and there appears to be a quantitative correlation between the extent of CPZ binding to CaM and the extent of inhibition of gravitropism (19).

We have investigated the potential involvement of CaM in the gravitropic response of primary roots of maize by: (a) measuring the distribution of CaM activity in roots, (b) comparing CaM activity in primary roots of dark-grown and light-grown seedlings of the cultivar Merit, in which gravitropic sensitivity is known to require illumination, and (c) comparing the time course of light-induced changes in CaM activity with the development of gravitropic sensitivity in roots of dark-grown seedlings exposed to light.

MATERIALS AND METHODS

Plant Materials. Maize seedlings (Zea mays L., B73 × Missouri 17, Mike Brayton Seeds, Ames, IA, or Merit, Asgrow Seed Co., Kalamazoo, MI) were raised as described by Mulkey et al. (17). The seedlings were used when the primary roots were approximately 1.5 cm long (about 3 d after planting). For experiments requiring dark-grown seedlings the seedlings were raised in the dark. For experiments on light-induction of gravitropic sensitivity in dark-grown seedlings the seedlings were raised in the dark for about 3 d and then exposed to fluorescent room lighting for specified periods prior to experimentation.

Crude Calmodulin Extracts. Crude CaM extracts were prepared by Tsoi et al. (29).

2 Abbreviations: EGTA, ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid; CaM, calmodulin; cAMP, cyclic adenosine monophosphate; CMZ, calmidazolium; CPZ, chlorpromazine; DCCPIP, 2,6-dichlorophenolindophenol; 5'-Nuc, 5'-nucleotidase; G-6-P, glucose 6-phosphate; PDE, phosphodiesterase; PDE buffer, phosphodiesterase assay buffer; PMS, phenazine methosulfate; TFP, trifluoperazine; Tricine, N-tris(hydroxymethyl)methyl-glycine.

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pared by homogenizing maize root tissue (1–2 cm apical segments) in 3 volumes per weight of 50 mM Tris-HCl 10 mM dithiothreitol (pH 8.0). The homogenate was heated to 85 to 90°C and then placed on ice and rapidly cooled to room temperature. The resulting mixture was centrifuged at 10,000g. The pellet was rehomogenized in 3 volumes of buffer, and the homogenate was centrifuged as above. The supernatants were combined and used as the crude CaM extract.

**Purified Extracts.** Apical root segments (300g, 1.5–2.0 cm) were homogenized in an equivalent volume of extraction buffer (20 mM Tris-HCl, 1 mM EDTA, 20 mM β-mercaptoethanol, 0.01% NaN₃, pH 7.5). The homogenate was filtered through 50 μm nylon mesh and the debris was rehomogenized in an additional one-half volume of extraction buffer. The filtrates were combined and centrifuged at 15,000g for 30 min. The supernatant was brought to 40% saturation with granulated ammonium sulfate and centrifuged at 20,000g for 30 min. The supernatant from this centrifugation was brought to 70% saturation with (NH₄)₂SO₄ and centrifuged again at 20,000g for 30 min. The pellet, representing the 40 to 70% (NH₄)₂SO₄ precipitable fraction, was resuspended in gel application buffer (300 mM NaCl, 1 mM CaCl₂, 50 mM Tris, 1 mM β-mercaptoethanol, 0.01% NaN₃, pH 7.5) and dialyzed against the same buffer for 24 h with one buffer change and a sample to buffer ratio of 0.01.

The dialyzed material was then heated by immersion in a boiling water bath. When the temperature of the sample reached 80°C, it was immediately transferred to an ice bath and cooled with rapid stirring until the temperature was below 10°C. Denatured protein was removed by centrifugation at 20,000g for 1 h. The supernatant was then applied at a flow rate of 0.8 ml min⁻¹ to a 0.7 × 4.5 cm phenothiazine-affigel column (Bio-Rad Laboratories) previously equilibrated with gel application buffer. The first 40 ml of eluate was reappied to the column and the column was washed with application buffer until the 280 nm absorbance of the eluate declined to within 0.05 A₂₈₀ of the initial preload baseline. The column was then eluted with elution buffer (20 mM Na₂EDTA, 50 mM Tris, 0.01% sodium azide, pH 7.5) while monitoring absorbance. Fractions containing protein were combined and dialyzed 24 h against application buffer prepared without NaCl (sample to buffer ratio 0.05).

The dialyzed samples were assayed for protein using the Bradford assay (5) and for CaM using the PDE assay. Sample homogeneity was assessed using SDS-PAGE. Samples containing CaM were dialyzed overnight (two changes of buffer) against double distilled water adjusted to pH 7.5 with 10-fold concentrated application buffer prepared without NaCl. The dialyzed samples were lyophilized and resuspended in a minimum volume of PDE buffer (7.5 mM Tris-HCl, 0.375 mM CaCl₂, 0.75 mM magnesium acetate, 0.75 mM imidazole, 25% w/w glycerol, pH 7.0).

**NAD Kinase Extraction.** NAD kinase was purified from 14-d-old pea (Pisum sativum L. var Willit Wonder) seedlings by the method of Harmon et al. (10). The enzyme was stored frozen (−80°C) in 0.5 ml aliquots.

**Gel Electrophoresis of Calmodulin Samples.** The purity of CaM extracts was assayed using 6 to 20% SDS-PAGE following the gel formulation of Laemmli (12). Gels were stained using the silver stain method of Merril et al. (16). Since CaM does not stain upon initial exposure the gels were routinely destained (6 g/L potassium dichromate, 2 ml/L 1.97% H₂SO₄) with destaining arrested by sodium sulfite (30 g/L). The destained gels were then thoroughly washed prior to staining. Remaining brought out the CaM bands intensely.

**Phosphodiesterase assay for Calmodulin.** A modified PDE assay similar to that described by Sharma and Wang (21) was used for determination of CaM. The assay solution consisted of 0.1 ml of assay buffer (360 mM Tris base, 360 mM imidazole, 45 mM magnesium acetate, pH 7.5), 20 μl of 4.5 mM CaCl₂, 20 μl of 5' Nuc (0.125 units/10 μl), and 2.5 μl of PDE (16 μM units/10 μl). Protein sample (10 μl) and 0.7 ml of water were added to the assay solution and the sample was mixed in a 1.5 ml Snap-Cap vial by vortexing briefly and then preincubated for 1 min at 30°C. The assay was started by adding 50 μl of 10.8 mM 3'5' cAMP and incubating for 30 min at 30°C. The assay was terminated by adding 100 μl of 55% TCA and vortexing. Precipitable material was removed by centrifugation for 10 min at 10,000g. Two aliquots of 0.4 ml each were removed and added to 200 μl of ammonium molybdate reagent (1.25 g/100 ml of 0.625 N sulfuric acid). The determination of CaM was initiated by adding 0.1 ml freshly prepared Fiske-Subbarow reagent (0.794 g/10 ml water) and incubating the sample aliquots at room temperature for 10 min. The absorption of the sample was measured at 660 nm using a Beckman model 25, UV/visible dual beam spectrophotometer. Each assay set included a blank containing all reagents except CaM. The amount of PDE utilized in the assay (4 m units/10 μl sample) was less than that used by Sharma and Wang (21) with comparable reduction of other reagent volumes. The unit of maize CaM activity is defined and was measured by the method of Sharma and Wang (21) using our assay conditions.

**Protein content was determined using a modification of the Bradford procedure (5). The Bradford reagent was used at a concentration 5-fold higher than originally reported (5) and was mixed directly with the sample (0.1 ml of concentrated Bradford reagent and 0.4 ml sample). All assays were performed with at least three replicates and at least one protein blank. Protein content is expressed as BSA equivalents.

**NAD Kinase Assay for Calmodulin.** The NAD kinase assay was performed essentially as described by Harmon et al. (10). The reaction was begun by adding 100 μl of CaM-containing sample to 0.5 ml of the assay solution (50 mM Tricine, 5 mM MgCl₂, 2 mM NAD, 3 mM ATP, 1 mM CaCl₂, pH 8.0) followed by the addition of 10 μl of the NAD kinase extract. The reaction mixture was allowed to incubate at 30°C for 30 min and then the reaction was terminated by placing the test tubes in a boiling water bath for 2 min. Following the heat treatment, 0.5 ml of a solution containing 50 mM Tricine (pH 8.0), 5 mM MgCl₂, 1 mM EDTA, 0.8 mM G-6-P, 0.2 mg/ml PMS, and 0.3 mg/ml DCPIP was added. The solution was transferred to a microcentrifuge cuvette and 20 μl of 6 units/ml G-6-P dehydrogenase was added. The rate of change in absorbance at 600 nm was monitored with a Beckman model 25 UV/visible dual beam spectrophotometer. Two controls were used, one containing EGTA and the other lacking NAD kinase. Calculations of units of CaM activity and measurement of protein was done as with the PDE assay.

**Chemicals.** The phenothiazine affinity gel was purchased from Bio-Rad Laboratories. Bovine CaM was purchased from Bio-Ringer-Mannheim Biochemicals, Indianapolis. All other chemicals were purchased from Sigma Chemical Co.

**RESULTS**

**Gel Electrophoresis of Purified Calmodulin.** Figure 1 shows an SDS-PAGE gel of crude extracts of maize roots before and after purification by phenothiazine affinity gel chromatography. Most of the protein extracted from the tissue was not retained by the column. The material retained by the column included about six bands as revealed by the modified Merril silver staining procedure. The major band (CaM) migrated at a position slightly ahead of purified bovine CaM, consistent with reports that plant CaM has a mol wt somewhat smaller than that of bovine brain CaM (11, 25). The less intense bands of higher mol wt retained by the column could be eliminated by passing the affinity-purified material through the column a second time. When this was done and the retained material was eluted from the column only one band was observed (data not shown).
Assay of Purified Calmodulin by Phosphodiesterase Activation. Figure 2 illustrates the comparative activity of purified maize root CaM and bovine brain CaM in the activation of PDE. Maize root CaM and bovine brain CaM are similar in their ability to activate PDE although maximal stimulation by maize root CaM is obtained at a somewhat lower concentration.

Assay of Intact Root Segments by Phosphodiesterase Activation and NAD Kinase Activation. There is evidence that a portion of the CaM in maize root tissue is releasable by osmotic shock (11). This suggests the possibility that a portion of maize root CaM is accessible to apoplastic constituents. We tested this idea by supplying intact tips as the CaM source in enzyme-activation assays. In both the PDE and NAD kinase assays strong calcium-dependent activation of enzyme activity was observed using maize root tips in place of purified CaM (Tables I and II). Enzyme activation by twenty 1-mm root tips was similar to that induced by 2.5 units × ml⁻¹ of purified CaM and was readily reversible in the presence of 10 mM EGTA.

Assay of Crude Calmodulin Extracts by Phosphodiesterase Activation and NAD Kinase Activation. In order to determine whether or not CaM could be reliably assayed in crude extracts from maize root tissue, CaM activity in partially purified extracts of maize roots was determined using both the PDE activation assay and the NAD kinase activation assay. For these experi-

**Table 1. Comparative Stimulation of Phosphodiesterase by Bovine Brain Calmodulin and Excised Maize Root Tip Tissue**

<table>
<thead>
<tr>
<th>Medium</th>
<th>2.5 units·ml⁻¹</th>
<th>Root Tip</th>
<th>Activity</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td>+</td>
<td>−</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>+EGTA</td>
<td>−</td>
<td>+</td>
<td>103</td>
<td>7</td>
</tr>
<tr>
<td>Complete</td>
<td>−</td>
<td>+</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>+EGTA</td>
<td>−</td>
<td>+</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>− cAMP</td>
<td>−</td>
<td>+</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>− 5' Nuc</td>
<td>−</td>
<td>+</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>− PDE</td>
<td>−</td>
<td>+</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

*Twenty 1-mm apical root segments (cv Merit, light grown).*
Table II. Comparative Stimulation of NAD Kinase by Bovine Brain Calmodulin and Excised Maize Root Tip Tissue

CaM activity is expressed as a percent of the NAD kinase activity in complete medium containing bovine brain CaM. Assays were performed in complete medium, complete medium plus 10 mM EGTA, or medium lacking one major constituent of the assay.

<table>
<thead>
<tr>
<th>Medium</th>
<th>CaM 2.5 units ml⁻¹</th>
<th>Root Tip*</th>
<th>Activity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td>+</td>
<td>–</td>
<td>100</td>
</tr>
<tr>
<td>+EGTA</td>
<td>+</td>
<td>–</td>
<td>0</td>
</tr>
<tr>
<td>Complete</td>
<td>–</td>
<td>+</td>
<td>90</td>
</tr>
<tr>
<td>+EGTA</td>
<td>–</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>– ATP</td>
<td>+</td>
<td>–</td>
<td>0</td>
</tr>
<tr>
<td>– ATP</td>
<td>–</td>
<td>+</td>
<td>15</td>
</tr>
<tr>
<td>– NAD</td>
<td>+</td>
<td>–</td>
<td>0</td>
</tr>
<tr>
<td>– NAD</td>
<td>–</td>
<td>+</td>
<td>5</td>
</tr>
<tr>
<td>– G6-P</td>
<td>+</td>
<td>–</td>
<td>0</td>
</tr>
<tr>
<td>– G6-P</td>
<td>–</td>
<td>+</td>
<td>15</td>
</tr>
<tr>
<td>– G6-PD</td>
<td>+</td>
<td>–</td>
<td>0</td>
</tr>
<tr>
<td>– G6-PD</td>
<td>–</td>
<td>+</td>
<td>13</td>
</tr>
<tr>
<td>– PMS</td>
<td>+</td>
<td>–</td>
<td>0</td>
</tr>
<tr>
<td>– PMS</td>
<td>–</td>
<td>+</td>
<td>15</td>
</tr>
</tbody>
</table>

* Twenty 1-mm apical root sections (cv Merit, light grown).

Table III. Inhibition of in Vitro Phosphodiesterase and NAD Kinase Activation by Calmodulin Antagonists

The CaM antagonists, TFP, CPZ, and CMZ were added to the assay media for measurement of activation of PDE and NAD kinase by extracts from twenty 1-mm root tips. CaM activity expressed as percent PDE or NAD kinase activity in complete medium containing extracts of root segments in the absence of inhibitors. The calmodulin activity of extracts of twenty 1-mm root segments was equivalent to 1.89 (PDE) and 1.56 (NAD kinase) units of bovine CaM x ml⁻¹, respectively.

<table>
<thead>
<tr>
<th>Medium</th>
<th>PDE Activity</th>
<th>NAD Kinase Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Complete + 10 μM TFP</td>
<td>46</td>
<td>26</td>
</tr>
<tr>
<td>Complete + 10 μM CPZ</td>
<td>40</td>
<td>32</td>
</tr>
<tr>
<td>Complete + 1 nM CMZ</td>
<td>37</td>
<td>32</td>
</tr>
</tbody>
</table>

Proteins crude extracts of maize root tissue were prepared as described in “Materials and Methods.” The extracts were tested for calcium-dependent activation of PDE or NAD kinase (Table III). Crude extracts of CaM strongly enhanced both PDE and NAD kinase activity, and in both cases the activity was reversible by 10 mM EGTA (data not shown). Low concentrations of the CaM antagonists TFP (10⁻⁴ M), CPZ (10⁻⁵), and calmidazolium (1 nM) strongly inhibited enzyme activity (Table III).

Distribution of Calmodulin Activity in Maize Root Tips. Our results indicate that the action of intact root sections in enhancing PDE and NAD kinase activity roughly parallels the CaM content of the tissue. We therefore used this method to estimate CaM activity as a function of position within the root (Fig. 3). The greatest CaM activity was found in the apical mm, including the root cap. Calmodulin activity in this region was several times greater than that of sections from the region one to four mm behind the tip.

Effect of Light on Calmodulin Activity in Roots of the Merit Cultivar of Maize. The primary roots of seedlings of the Merit cultivar of maize are gravisensitive in light-grown but not in dark-grown seedlings (8). In contrast, the gravisensitivity of primary roots of seedlings of the cultivar B73 × Missouri 17 remains unaffected by light. Since illumination of the cap is reported to enhance both gravitropic sensitivity and the synthesis of certain proteins in the cap (8), we investigated whether or not the CaM activity in the root cap of the Merit cultivar is influenced by light. This was examined by growing seedlings of the Merit and Missouri cultivars either in complete darkness or under fluorescent room lighting. After 3 d the root tips (first 1 mm) were excised and combined in lots of 20. The CaM activity of the sections was assayed either in the intact sections or in crude extracts of the sections using both the PDE and NAD kinase assays (Table IV). Although the PDE activity in root tips of the Missouri hybrid was high in both light-grown and dark-grown seedlings, there was a 3-fold difference in the CaM activity of dark-grown and light-grown seedlings of Merit with the CaM activity higher in root tips from light grown seedlings.

Comparative Time Course of Photoenhancement of CaM Activity and Gravisensitivity in Roots. In order to compare the time courses of photoenhancement of CaM activity and gravisensitivity, we analyzed CaM activity and gravitropic sensitivity in root tips of dark-grown seedlings of the Merit cultivar of maize as a function of time following the beginning of illumination. As shown in Figure 4 the time course for photoenhancement of CaM activity parallels, but precedes by about 40 min the time course for photoinduction of gravitropic competence. Similar results were obtained when CaM was assayed in crude extracts from root caps (fig. 5).

DISCUSSION

The results indicate that CaM is present in the apical portion of the primary root of maize seedlings. Calmodulin activity was assayable in extracts from root tissue using either the PDE activation assay or the NAD kinase activation assay. In addition, a protein extracted from the roots was retained by CaM phenothiazine affinity gel chromatography and this protein co-chromatographed with authentic CaM on SDS-PAGE gels.
CALMODULIN AND ROOT GRAVITROPISM

The NAD kinase assay is reported to be extremely sensitive and specific for CaM. Harmon et al. (10) reported 50% activation of NAD kinase by CaM in the range of 0.520 to 2.2 ng/ml whereas comparable activation of PDE required CaM concentrations in the range of 19.6 to 73.5 ng/ml. The NAD kinase assay for CaM was found to be more sensitive than radioimmunoassay and not subject to nonspecific enzyme activation by phospholipids or fatty acids. Unlike other CaM-activated enzymes, NAD kinase is also not activated by partial proteolysis. Also, estimates of the CaM content of crude homogenates were found to be consistent with estimates obtained after various purification procedures.

We were surprised to find that strong CaM activity was detectable in maize root sections simply by using the sections as the source of CaM in either the PDE or NAD kinase assay. There are a number of potential explanations for this observation: (a) the root sections may supply some component other than CaM that enhances enzyme activity, (b) calmodulin may be released from broken cells when the sections are cut, (c) calmodulin may be released as cells slough off from the root cap and accumulate in the mucilage, (d) the CaM activity may be from microflora associated with the root surface, (e) CaM may be associated with the plasma membrane and accessible to apoplastic constituents, and (f) CaM may be secreted into the apoplast.

The results in Tables I and II indicate that the CaM activity observed in intact tissue segments cannot be attributed to release from the segments of some rate-limiting component of the assay or to direct supply of the assayed end product (e.g. phosphate in the case of the PDE assay, direct reduction of PMS or dichlorophenolindophenol in the NAD kinase assay). In the presence of CaM root segments were unable to enhance assayed enzyme activity in media lacking any one of the major assay components, indicating that the roots do not provide rate-limiting assay constituents other than CaM. Furthermore, most of the enzyme activity attributable to the root segments was reversible by EGTA, suggesting a calcium-dependent activation mechanism.

Release of CaM from cells broken during cutting cannot be ruled out as a factor contributing to whole segment CaM activity in the enzyme activation assays. However, this seems unlikely to be a major factor since the activity was not reduced by washing the segments prior to the assay and it was not increased substantially by subsectioning the tissue sample into smaller portions (data not shown). Likewise it seems unlikely that the CaM activity of intact root segments is attributable to cells sloughed off from the root cap into the mucilage. CaM activity remained high after wiping the mucilage from the most apical segment, and segments in the region 3 to 4 mm behind the tip would not be expected to contain such cells. The possibility that the CaM activity of the segments is due to microflora on the surface of the root was tested by raising seedlings with the roots growing in an antibiotic/antimycotic solution. This did not affect the CaM activity of segments taken from the roots (data not shown). This, in conjunction with the fact that CaM has not been found in prokaryotes, suggests that rhizosphere microflora do not account for the CaM activity of intact root sections.

The remaining alternatives for explaining the CaM activity of whole tissue segments assume the presence of CaM in the apoplast or the occurrence of CaM in association with the plasma membrane in a manner rendering it accessible to apoplastic constituents. While our data do not prove the existence of apoplastic CaM, there are other indirect indications for its occurrence there. Kuzmanoff (11) was able to release CaM from corn root tissue using osmotic shock, and Biro et al. (4) detected CaM in the cell walls of oat coleoptile tissue using radioimmunoassay.

Even though the source of CaM activity measurable in whole root sections is uncertain, the fact that this activity is readily detectable provides a useful tool for comparative measurements of tissue CaM activity. We found whole segment CaM activity to be quantitatively proportional to total extractable activity. Thus, whole segment assays can be used to measure relative CaM activity in various regions of the root. Using this approach, we found CaM activity to be about 3-fold higher in the apical mm of the root compared with the zone 1 to 4 mm behind the tip. Similar results were reported by Allan and Treewavas (1) for CaM distribution in the root apex of Pisum sativum. It is likely that the higher level of CaM found in the apical mm is due in part to the greater density of protoplasm there as compared with cells in the elongation zone. However, this does not appear to account entirely for the difference since the CaM level of the apical mm was found to be 3-fold greater than that of the 1 mm sections from the elongation zone even when CaM content was calculated on a per μg protein basis.

The light-dependence of CaM activity in the apical mm of the root is particularly intriguing. Light causes a rapid increase in the CaM activity in the apical mm of the primary root of dark-
grown seedlings of the Merit cultivar of maize. The increase in CaM activity precedes light-induced gravitropic responsiveness in these roots. There are other indications of light-mediated quantitative changes in CaM. Zielinski (26) found 2- to 3-fold higher levels of Cam mRNA and protein in the meristematic region of barley leaves, compared with the levels found in regions of the leaf lacking dividing cells. He reported that, during light-induced leaf development in dark-grown seedlings, there was a 2- to 4-fold decline in translatable CaM mRNA. The light-dependent decrease in mRNA was detectable within 30 min and occurred with a half-time of about 1 h. L.J Feldman (personal communication) has recently found rapid (within 10 min) light-induced increases in CaM mRNA in root caps of dark-grown seedlings of the Merit cultivar of maize. This is consistent with our finding of rapid light-induced increases in CaM activity in this tissue. In a recent study (24) Vega and Boland found that vitamin D-3 induces de novo synthesis of CaM in bean (Phaseolus vulgaris) root segments and they speculated that light-induced synthesis of vitamin D-3 (and hence CaM) might mediate certain light effects on roots.

The correlation between light effects on gravitropic sensitivity and CaM activity in maize roots suggests the possibility of a role for CaM in root gravitropism. Although this suggestion is based upon indirect correlative information, it is consistent with the observation that root gravitropism is closely linked to gravity-induced polar calcium movement (15) and with the finding that CaM antagonists inhibit both shoot (2) and root (23) gravitropism.

LITERATURE CITED