Plant Physiol. (1982) 70, 162–167
0032-0889/82/70/0162/06/$00.50/0

Ethylene Biosynthesis and Cadmium Toxicity in Leaf Tissue of Beans (*Phaseolus vulgaris* L.)[1]

Received for publication October 21, 1981 and in revised form March 6, 1982

JÜRG FUHRER[2]
School of Forestry and Environmental Studies, Yale University, New Haven, Connecticut 06511

ABSTRACT

Stress ethylene production in bean (*Phaseolus vulgaris* L., cv. Taylor's Horticultural) leaf tissue was stimulated by Cd^{2+} at concentrations above 1 micromolar. Cd^{2+}-induced ethylene biosynthesis was dependent upon synthesis of 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase. Activity of ACC synthase and ethylene production rate peaked at 8 h of treatment. The subsequent decline in enzyme activity was most likely due to inactivation of the enzyme by Cd^{2+}, which inhibited ACC synthase activity *in vitro* at concentrations as low as 0.1 micromolar. Decrease in ethylene production rate was accompanied by leakage of solutes and increasing inhibition of ACC-dependent ethylene production. Ca^{2+}, present during a 2-hour preincubation, reduced the effect of Cd^{2+} on leakage and ACC conversion. This suggests that Cd^{2+} exerts its toxicity through membrane damage and inactivation of enzymes. The possibility of an indirect stimulation of ethylene biosynthesis through a wound signal from injured cells is discussed.

Ethylene is an endogenous regulator for plant growth, development, and senescence (1). Ethylene production from plants under normal conditions is very low, but increases when living tissues are subjected to a variety of stress conditions (21). Biosynthesis of ethylene begins with methionine and includes SAM[3] and ACC as intermediates (3). Formation of ACC by ACC synthase has been identified as rate-limiting step in vegetative and fruit tissue (6, 7, 15, 17, 33, 34, 36). This step can be inhibited specifically by AVG (3, 6, 8, 34). The mechanisms by which ACC synthase activity is regulated are yet unknown.

Conversion of ACC to ethylene presumably involves a membrane associated system (4, 26). Osmotic shock (4, 26, 27), low temperature (4, 11, 27), high temperature (12, 16, 34), lipophilic membrane perturbants (4, 24, 25), ionic and nonionic detergents (3, 4, 26, 27), and polyamines (5) are known to inhibit this system. Cobaltous ion (19) and high phosphate concentrations (9) were used during the experiments reported here to inhibit this step. Stress ethylene production from plants exposed to toxic chemicals such as copper (18) or sulfur dioxide (28) declines when the tissue shows toxicity symptoms. In contrast, ethylene production induced by nontoxic levels of mercury vapor does not decrease (14). This suggests that toxic chemicals have a dual effect on ethylene biosynthesis, stimulation, and inhibition.

Experiments were undertaken to determine the step at which toxic heavy metals exert their effects on the biosynthetic pathway of ethylene and through which mechanism stimulation and inhibition occur. Cadmium ion has been chosen because of its increasing importance as an environmental contaminant. Although the phytotoxic potential of Cd^{2+} is well known (29), more information about specific effects of this trace element on biochemical processes in plants is needed.

MATERIALS AND METHODS

Chemicals. ACC was purchased from Sigma, and Hepps from P-L Biochemicals Inc. AVG was a gift from Hoffmann-La Roche Inc.

Plant Materials. Beans (*Phaseolus vulgaris* L., cv. Taylor's Horticultural) were grown in vermiculite in an environmental growth chamber with a 16-h photoperiod at 25°C and 55% RH. Fully expanded trifoliar leaves of 30- to 32-d old plants were used for all experiments.

Incubation. Leaf discs (12 mm in diameter) were cut with a corkborer and floated abaxial side down under white fluorescent light for 2 h in Petri dishes containing 1 mM Na-phosphate buffer (pH 5.9), chloramphenicol (50 μg/ml), and effectors as indicated for each experiment. This procedure, which is referred to as preincubation, was necessary, because Cd^{2+} was found to inhibit uptake of these effectors (unpublished observation). At the end of the preincubation period, 5 discs were transferred to 70-ml flasks containing 5 ml buffer (with chloramphenicol), Cd^{2+} and effectors as indicated. The flasks were flushed with air, sealed with a rubber serum cap, and incubated at 25°C in darkness. Each experiment was repeated three times and consisted of three replicates. The presented data are means of three replicates of a representative experiment.

Ethylene Determination. Gas samples were withdrawn from the flasks at indicated times and injected into the GC (model F-11; Perkin Elmer) equipped with an activated alumina column. Ethylene was identified and quantified in comparison to the retention time and peak height of ethylene standards.

Assay of ACC. ACC was extracted at indicated times according to Bufler *et al.* (8) with slight modifications. Leaf tissue (0.3 g fresh weight) was ground in liquid N_2, then extracted with 3 ml ethanol (80%, v/v) for 16 h at 4°C with constant shaking. The extract was centrifuged at 10,000*g* for 15 min, resuspended in 3 ml ethanol, and recentrifuged as above. Combined supernatants were dried at 37°C in a N_2 stream, and residues were dissolved in a known amount of H_2O. After centrifugation at 2,000*g* for 10 min, ACC content in the aqueous solution was determined according to Lizada and Yang (22). Addition of Cd^{2+} (up to 1 mmol/l) to the final solution did not disturb the ACC determination.

Assay of ACC Synthase. Preparation and assay of ACC synthase were in accordance to Yu and Yang (36), Boller *et al.* (6), and J. Riov (personal communication). Leaf discs (approximately

[1] Supported by a Swiss National Science Foundation Postdoctoral Fellowship.

[2] Present address: Institute of Plant Physiology, University of Bern, Altenbergrain 21, CH-3013 Bern, Switzerland.

[3] Abbreviations used: SAM, *S*-adenosylmethionine; ACC, 1-aminocyclopropane-1-carboxylic acid; AVG, amino-ethoxyvinylglycine; CHI, cycloheximide; Hepps, 4-(2-hydroxyethyl)-1-piperazinepropanesulfonic acid.

1 g fresh weight) were thoroughly washed with H_2O and homogenized in 3 ml K-Hepps buffer (50 mM, pH 8.5) containing 0.5 μM pyridoxal phosphate and 4 mM DTT. The homogenate was centrifuged at 10,000*g* for 20 min. Proteins in the supernatant were precipitated by slow addition of ammonium sulfate (90% of saturation) under gentle stirring for 2 h at 4°C. After centrifugation (15,000*g*, 20 min), the pellet was dissolved in 1 ml of dialysis solution, containing 2 mM K-Hepps (pH 8.5), 0.5 μM pyridoxal phosphate, and 0.1 mM DTT. Dialysis was carried out over night. ACC synthase activity was determined in a reaction mixture containing 0.4 ml enzyme solution, 50 μl K-Hepps (600 mM, pH 8.5), 90 μl H_2O, and 60 μl SAM (0.6 mM). ACC content in the reaction mixture was measured after 1 or 2 h of incubation at 30° C by the method of Lizada and Yang (22). Reaction rate was constant during this time period. Tests with blank samples were performed in parallel. They contained 0.4 ml enzyme solution

Table I. *Comparative Effect of Different Heavy Metals on Ethylene Production and Leakage of Solutes from Bean-Leaf Discs*

All metals were added as their chlorides (0.1 mM) except for Hg°. Mercury vapor was originated from 1 ml of Hg metal contained in a small vial which was placed into the 70-ml incubation flask according to Goren and Siegel (14). The Hg° level was that of air saturated at 25°C (0.007 μmol/l). Samples consisted of five discs floating on 5 ml of incubation solution. Measurements were carried out after a 17-h incubation. Values in one column followed by the same letter are not significantly different at the 5% level of probability.

	Ethylene	Leakage
	nmol/g·17 h	A_{280}
Control	2.1 c	0.04 c
Cd^{2+}	209.3 a	0.65 a
Cu^{2+}	6.6 b	0.25 b
Hg^{2+}	4.1 bc	0.21 b
Zn^{2+}	3.3 bc	0.16 bc
Hg°	1.6 d	0.12 c

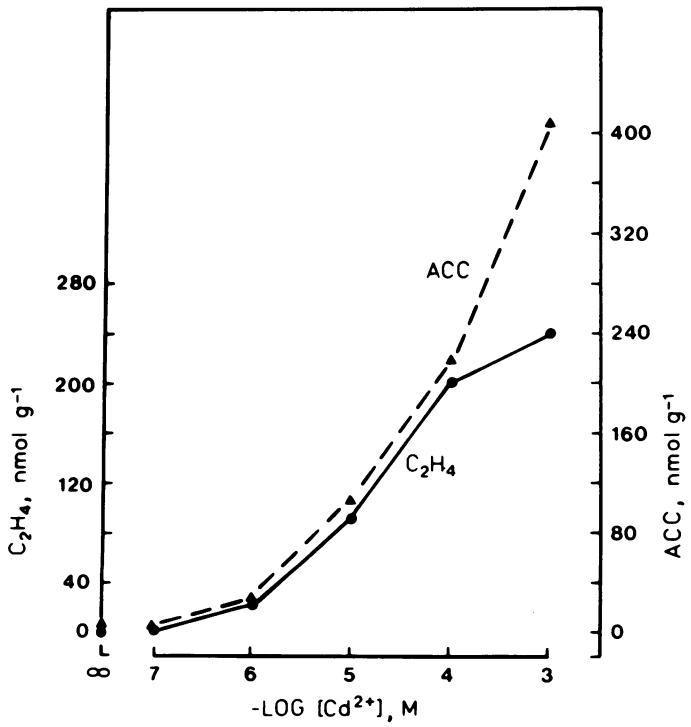

FIG. 1. Effect of various Cd^{2+} concentration on ACC formation and ethylene (C_2H_4) production by bean leaf tissue. Accumulation of ethylene and ACC content were determined after a 17-h incubation period.

Table II. *Effect of Cycloheximide, AVG, Phosphate, and Ca^{2+} ion on Cd^{2+}-Induced ACC Formation and Ethylene Production from Bean-Leaf Discs*

Measurements were carried out after a 17-h incubation on 0.1 mM Cd^{2+}. The effectors were present during a 2-h preincubation and the incubation on Cd^{2+}. Values in one column followed by the same letter are not significantly different at the 5% level of probability.

	Ethylene	ACC
	nmol/g·17 h	*nmol/g*
Control	2.1 d	1.6 d
Cd^{2+}	209.3 a	220.5 d
Cd^{2+} + CHI, 0.01 mM	3.1 d	2.9 d
Cd^{2+} + AVG, 20 μM	13.4 c	12.6 d
Cd^{2+} + Ca^{2+}, 2 mM	13.7 c	157.0 b
Cd^{2+} + HPO_4^{2-}, 100 mM	4.4 cd	47.9 c

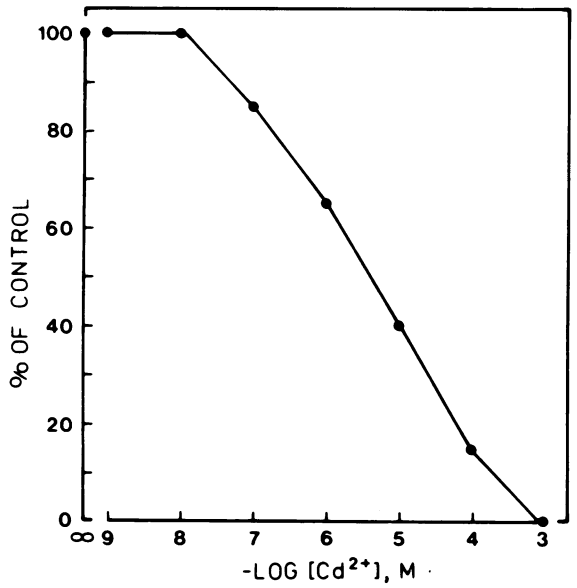

FIG. 2. Effect of various Cd^{2+} concentrations on the *in vitro* activity of ACC synthase. ACC synthase was extracted from bean-leaf discs which had been incubated for 6 h on 0.1 mM Cd^{2+} (100% represents 10.2 nmol g^{-1} 2 h^{-1} of enzyme activity). Various Cd^{2+} concentrations were added to the assay mixtures.

which has been heated at 65°C for 15 min.

Determination of Solute Leakage. Leakage of solutes from the leaf discs during the incubation period was followed by measuring *A* at 280 nm (Aminco spectrophotometer, DW-2a) of the incubation solution at different times (20). Each sample consisted of five discs floating on 5 ml of solution. Samples were gently shaken before transferring an aliquot into a quartz cuvette. The aliquot was transferred back to the flask after the measurement to maintain a constant volume of the sample.

RESULTS

Ethylene production from untreated bean leaf discs was about 1% of that produced by Cd^{2+}-treated (0.1 mM) discs (Table I). In comparison, Cu^{2+} (0.1 mM) only slightly stimulated ethylene production under the described conditions, and Hg^{2+} and Zn^{2+} (both at 0.1 mM) had no significant effect. Hg vapor (at air saturation) was inhibitory. Similarly, leakage of solutes was markedly stimulated by Cd^{2+}, whereas the other ions tested were less effective (Table I). *A* at 280 nm was due predominantly to proteins (not shown).

Cd^{2+} concentrations above 1 μM were effective in stimulating

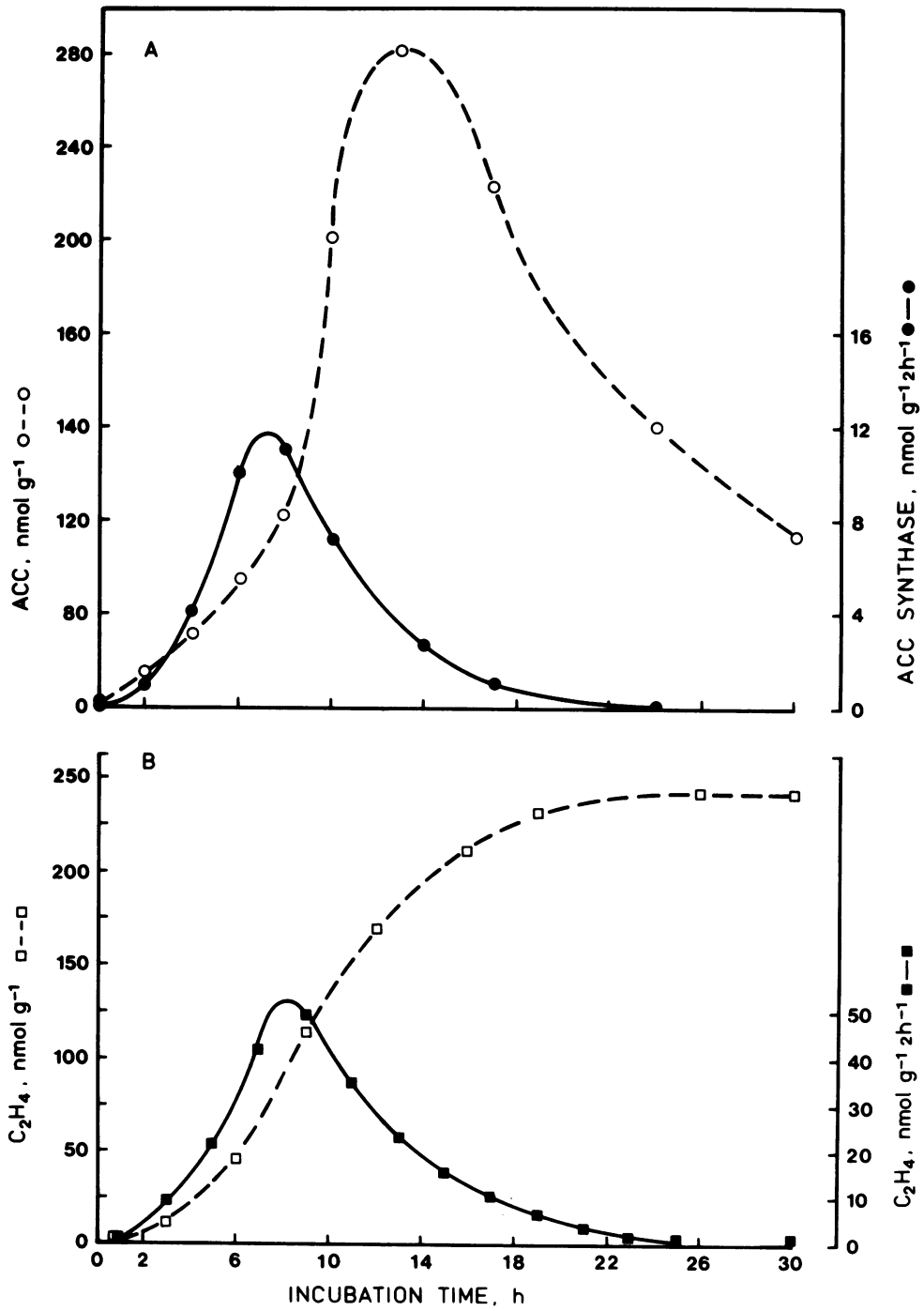

FIG. 3. A, Time courses of ACC synthase activity and ACC content; B, ethylene (C_2H_4) production by bean leaf tissue exposed to Cd^{2+} (0.1 mM). Each value for ethylene production represents the total amount of ethylene accumulated during the indicated incubation period. The curve for ethylene production rate has been calculated from the accumulation of ethylene during each individual 2-h incubation period.

ethylene production and formation of ACC, the precursor of ethylene (Fig. 1). A close relationship existed between ACC content and ethylene production from discs treated with Cd^{2+} up to 0.1 mM. ACC content was high, relative to ethylene production, in the case of 1 mM Cd^{2+}. The effect of several effectors, known to inhibit protein synthesis (CHI), ACC synthesis (AVG), or conversion of ACC to ethylene (Co^{2+}, HPO_4^{2-}) on Cd^{2+}-induced ethylene production was examined. The results are presented in Table II. Formation of ACC and ethylene production were effectively inhibited in the presence of AVG and CHI.

After preincubation on solutions containing Co^{2+} (2 mM) or PO_4^{2-} (100 mM), ethylene production during the following Cd^{2+} treatment was inhibited; however, ACC contents of the tissue were much higher than in the cases of AVG or CHI.

The effect of Cd^{2+} on ACC synthesis was tested *in vitro* and *in vivo*. The direct effect of Cd^{2+} on ACC synthase was examined by adding various Cd^{2+} concentrations in the enzyme assay. The results presented in Figure 2 demonstrate that already 0.1 μM Cd^{2+} inhibited ACC synthase activity by 15%.

Figure 3A shows the time courses of ACC synthase activity and ACC content during treatment with 0.1 mM Cd^{2+}. ACC content increased from 0.6 to 15.2 nmol/g over the first 2-h interval. ACC

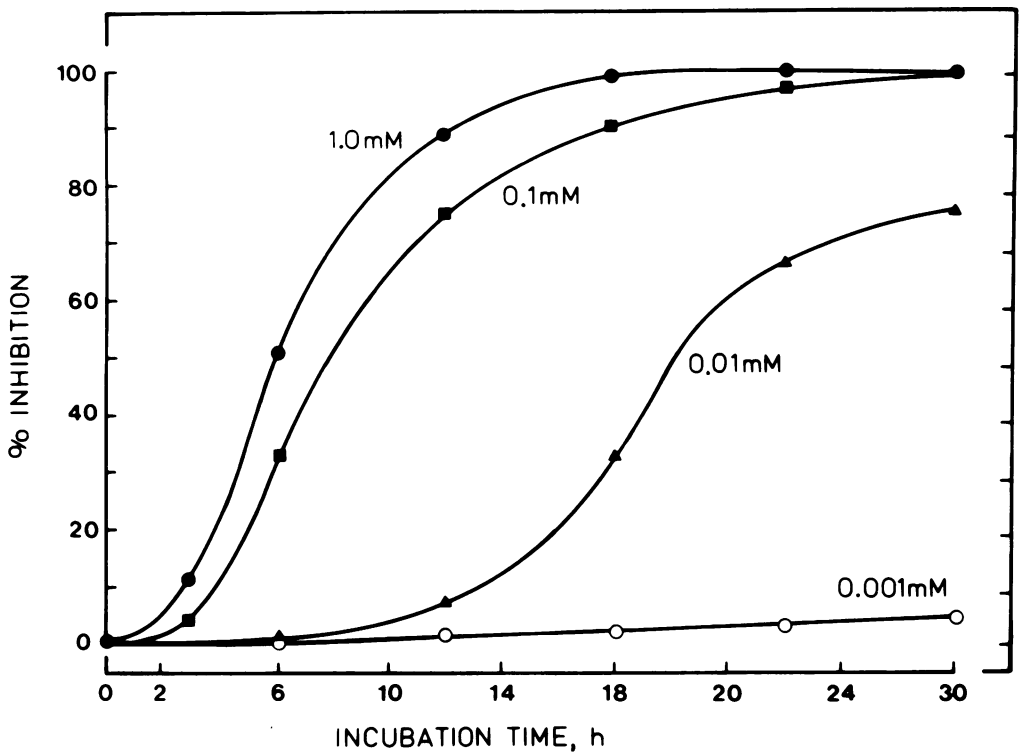

FIG. 4. Time course of inhibition of ACC-dependent ethylene production by various Cd^{2+} concentrations. Leaf discs were preincubated in 1 mM ACC and 20 μM AVG, and then transferred to solutions containing various Cd^{2+} concentrations and AVG. Each value has been calculated from the production of ethylene by control and Cd^{2+}-treated discs during the individual incubation intervals.

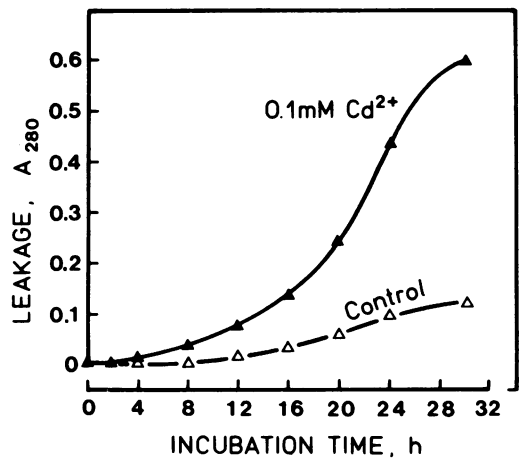

FIG. 5. Time course of leakage of solutes from bean-leaf discs incubated on H_2O (control) or 0.1 mM Cd^{2+}. Values represent UV-absorbance of the incubation solution (A_{280}) at indicated times. Each sample consisted of five discs floating on 5 ml solution.

synthase activity increased concurrently from 0 to 0.7 nmol/g·2h. Values for ACC synthase were lower than expected from ACC accumulation rate. A similar observation has been reported by Boller and Kende (7). The time courses of the total amount of ethylene formed after addition of 0.1 mM Cd^{2+} and of the calculated rate of ethylene production during 2-h intervals are shown in Figure 3B. Activity of ACC synthase and ethylene production rate peaked at 8 h and declined afterwards. ACC content, which depends on the rate of ACC synthesis and conversion, was highest at 13 h. Decline of ACC content following 13 h accompanied the rapid decrease in ACC synthase activity. The observed decrease in ethylene production rate at the same time, however, could not be attributed entirely to the decrease in ACC content. When

Table III. *Effect of Ca^{2+} on Ethylene Production Rate and Leakage of Solutes from Discs Treated with Cd^{2+}*

Ca^{2+} was present at 1 mM during the preincubation period. The concentration of Cd^{2+} was 0.1 mM. Each ethylene production value represents the accumulation during a 2-h interval (9–11 h and 19–21 h) per g fresh wt. Samples for determination of leakage consisted of five discs floating on 5 ml of incubation solution. Values in one column followed by the same letter are not significantly different at the 5% level of probability.

	10 h		20 h	
	Ethylene	Leakage	Ethylene	Leakage
	nmol	A_{280}	*nmol*	A_{280}
Control	0.3 b	0.01 b	0.3 c	0.06 c
Cd^{2+}, 0.1 mM	70 a	0.05 a	8 b	0.24 a
Cd^{2+} + Ca^{2+}	58 a	0.01 b	25 a	0.12 b

ethylene production stopped (at 24 h), ACC was still present in the tissue. Only negligible amounts of ACC were found in the incubation solution after 30 h of incubation. This ruled out that ACC content decreased because of leakage of ACC into the incubation medium. The possibility of a negative effect of Cd^{2+} on the ACC converting system was tested by adding ACC (1 mM) to the preincubation medium. The ACC-preincubated discs were treated with various concentrations of Cd^{2+} in the absence of exogenous ACC (in order to avoid interference at the uptake site), but in the presence of AVG to inhibit Cd^{2+}-induced ACC formation. Inhibition of ACC-dependent ethylene production by Cd^{2+} at 1.0, 0.1, or 0.01 mM increased progressively during the treatment (Fig. 4). It is noteworthy that Cd^{2+} did not disturb the assay for ACC content as described in "Materials and Methods" (data not shown).

Evidence for membrane damage as the cause for the decline in both, Cd^{2+}-induced and ACC-dependent ethylene production with time, was found by measuring leakage of solutes. Leakage

due to the cutting occurred during the preincubation period and did not disturb the measurement during the treatment. Figure 5 shows that increasing leakage (compared to the control) was found following 4 h of treatment with 0.1 mM Cd^{2+}. The presence of 1 mM Ca^{2+} reduced Cd^{2+}-induced leakage and decrease in ethylene production rate between 10 and 20 h (Table III). Leakage was not affected by AVG, indicating that internal ethylene was not causing disturbance of membrane integrity (data not shown).

DISCUSSION

The present study demonstrates that Cd^{2+} ions, known to be toxic to plants (29), interfere specifically with ethylene biosynthesis in bean leaf tissue. Treatments with concentrations above 1 μM initially stimulate ACC synthase activity, as measured *in vitro*. Enhanced activity of this key enzyme for ethylene biosynthesis (6, 33) is associated with an increased synthesis of ACC and ethylene. Inhibition of ACC synthesis by the specific ACC synthase-inhibitor AVG, and of ACC conversion by Co^{2+} or high phosphate concentrations suggests that Cd^{2+}-induced stress ethylene is derived from methionine, as is stress ethylene in other cases (2, 7, 10, 17, 36).

Stimulation of ACC synthase has also been found in response to mechanical wounding (7), IAA (15, 35), and Cu^{2+} (36). It is not clear whether such different stimuli regulate ACC synthase through common mechanism(s). The contradictory *in vitro* and *in vivo* effects of Cd^{2+} on ACC synthase activity indicate that the observed increase in enzyme activity during the first 8 h of Cd^{2+} treatment is an indirect effect. The direct contact between Cd^{2+} ions and the ACC synthase protein *in vitro* results in an inhibition of the enzyme activity. The decline in extractable ACC synthase *in vivo* following 8 h of Cd^{2+} treatment is therefore most likely due to the direct interaction between penetrating Cd^{2+} ions and the enzyme in the tissue. Similarly, Cd^{2+} has been found to stimulate glutamate dehydrogenase and malate dehydrogenase from beans *in vivo*, but to inhibit these enzymes *in vitro* (32).

It has been suggested that mechanical wounding of pea stems leads to the formation of a wound 'signal,' which in turn causes stimulation of ethylene biosynthesis (30). It may therefore be assumed that Cd^{2+}-induced production of ethylene by intact cells is a response to such a signal from adjacent cells which are directly affected by Cd^{2+} ions. Experiments are in progress to determine quick physicochemical changes in the tissue following initiation of the Cd^{2+} stress and their relationship to the induction of ACC synthase.

Cd^{2+} is known to exert toxic effects through reaction with SH-groups of proteins, thus inactivating numerous enzymes (23, 31, 32). This ability of Cd^{2+} may account for the inactivation of ACC synthase *in vitro* and *in vivo*, as well as for the decreased conversion of ACC to ethylene during the Cd^{2+} treatment. This last step in ethylene biosynthesis can be inhibited by sulfhydryl reagents, such as $CoCl_2$, $HgCl_2$, or others (13, 35). Recent work suggests the involvement of an intact membrane or cell wall-membrane system for ACC-dependent ethylene production (4, 11, 12, 26, 27).

Leakage of solutes reflects loss of membrane integrity (20). Leakage induced by Cd^{2+}, as observed during these experiments, therefore indicates that Cd^{2+} ions disturb membrane structures. As a possible result, the membranes affected by Cd^{2+} lose the ability to convert ACC to ethylene, or, alternatively, to transport ACC to the site of its conversion. In fact, ACC conversion to ethylene declines during treatment with Cd^{2+}-concentrations above 1 μM. The ability of Ca^{2+} to antagonize both leakage and decrease in ACC-dependent ethylene production underlines the link between integrity and functionality of cell membranes. Based on these observations, it may be concluded that ethylene biosynthesis can be regulated not only at the step of ACC synthesis, but also at the site of ACC conversion to ethylene. Interestingly, ACC content of the tissue further declines, after ethylene production is stopped. This raises the question about an ACC metabolite other than ethylene. The apparently destructive effect of Cd^{2+} on membranes and enzymes involved in important processes of cell regulation may be a reason for the high phytotoxic potential of this trace element.

Acknowledgments—I wish to thank Professor A. W. Galston and W. H. Smith for their support, Professor B. B. Stowe for his assistance with the GC, and Professor R. Goren for his constant interest and the critical reading of the manuscript.

LITERATURE CITED

1. ABELES FB 1973 Ethylene in Plant Biology. Academic Press, New York, pp 87–102
2. ABELES AL, FB ABELES 1972 Biochemical pathway of stress-induced ethylene. Plant Physiol 40: 496–498
3. ADAMS DO, SF YANG 1979 Ethylene biosynthesis: identification of l-aminocyclopropane-l-carboxylic acid as an intermediate in the conversion of methionine to ethylene. Proc Natl Acad Sci USA 76: 170–174
4. APELBAUM A, AC BURGOON, JD ANDERSON, T SOLOMOS, M LIEBERMAN 1981 Some characteristics of the system converting l-aminocyclopropane-l-carboxylic acid to ethylene. Plant Physiol 67: 80–84
5. APELBAUM A, AC BURGOON, JD ANDERSON, M LIEBERMAN 1981 Polyamines inhibit biosynthesis of ethylene in higher plant tissue and fruit protoplasts. Plant Physiol 68: 453–456
6. BOLLER T, RC HERNER, H KENDE 1979 Assay for and enzymatic formation of an ethylene precursor, l-aminocyclopropane-l-carboxylic acid. Planta 145: 293–303
7. BOLLER T, H KENDE 1980 Regulation of wound ethylene synthesis in plants. Nature (Lond) 286: 259–260
8. BUFLER G, MS REID, SF YANG 1980 Changes in l-aminocyclopropane-l-carboxylic acid content of cut carnation flowers in relation to their senescence. Planta 150: 439–442
9. CHALUTZ E, AK MATTOO, Y FUCHS 1980 Biosynthesis of ethylene: the effect of phosphate. Plant Cell Environ 3: 349–356
10. DE LAAT AMM, LC VAN LOON, CR VONK 1981 Regulation of ethylene biosynthesis in virus-infected tobacco. 1. Determination of the role of methionine as the precursor of ethylene. Plant Physiol 68: 256–260
11. FIELD RJ 1981 The effect of low temperature on ethylene production by leaf tissue of *Phaseolus vulgaris* L. Ann Bot 47: 215–223
12. FIELD RJ 1981 A relationship between membrane permeability and ethylene production at high temperature in leaf tissue of *Phaseolus vulgaris* L. Ann Bot 48: 33–39
13. GEPSTEIN S, KV THIMANN 1980 The effect of light on the production of ethylene from l-aminocyclopropane-l-carboxylic acid by leaves. Planta 149: 196–199
14. GOREN R, SM SIEGEL 1976 Mercury-induced ethylene formation and abscission in *Citrus* and *Coleus* explants. Plant Physiol 57: 628–631
15. JONES JF, H KENDE 1979 Auxin-induced ethylene biosynthesis in subapical stem sections of etiolated seedlings of *Pisum sativum* L. Planta 146: 649–656
16. KONZE JR, H KENDE 1979 Ethylene formation from 1-aminocyclopropane-l-carboxylic acid in homogenates of etiolated pea seedlings. Planta 146: 293–301
17. KONZE JR, GMK KWIATKOWSKI 1981 Rapidly induced ethylene formation after wounding is controlled by the regulation of l-aminocyclopropane-l-carboxylic acid synthesis. Planta 151: 327–330
18. LAU OL, SF YANG 1976 Stimulation of ethylene production in the mung bean hypocotyls by cupric ion, calcium ion, and kinetin. Plant Physiol 57: 88–92
19. LAU OL, SF YANG 1976 Inhibition of ethylene production by cobaltous ion. Plant Physiol 58: 114–117
20. LEOPOLD AC, ME MUSGRAVE 1979 Respiratory changes with chilling injury of soybeans. Plant Physiol 64: 702–705
21. LIEBERMAN M 1979 Biosynthesis and action of ethylene. Annu Rev Plant Physiol 30: 533–591
22. LIZADA MCC, SF YANG 1979 A simple and sensitive assay for l-aminocyclopropane-l-carboxylic acid. Anal Biochem 100: 140–145
23. MATHYS W 1975 Enzymes of heavy-metal-resistant and non-resistant populations of *Silene cucubalus* and their interaction with some heavy metals *in vitro* and *in vivo*. Physiol Plant 33: 161–165
24. MATTOO AK, JE BAKER, E CHALUTZ, M LIEBERMAN 1977 Effect of temperature on the ethylene-synthesizing systems in apple, tomato and *Penicillium digitatum*. Plant Cell Physiol 18: 715–719
25. MATTOO AK, E CHALUTZ, M LIEBERMAN 1979 Effect of lipophilic and water-soluble membrane probes on ethylene synthesis in apple and *Penicillium digitatum*. Plant Cell Physiol 20: 1097–1106
26. MATTOO AK, M LIEBERMAN 1977 Localization of the ethylene-synthesizing system in apple tissue. Plant Physiol 60: 794–799
27. ODAWARA S, A WATANABE, H IMASEKI 1977 Involvement of cellular membrane in regulation of ethylene production. Plant Cell Physiol 18: 569–575
28. PEISER GD, SF YANG 1979 Ethylene and ethane production from sulfur dioxide-injured plants. Plant Physiol 63: 142–145
29. RAUSER WE 1978 Early effects of phytotoxic burdens of cadmium, cobalt, nickel, and zinc in white beans. Can J Bot 56: 1744–1749
30. SALTVEIT ME JR, DR DILLEY 1978 Rapidly induced wound ethylene from excised segments of etiolated *Pisum sativum* L., cv. Alaska. III. Induction and

transmission of the response. Plant Physiol 62: 710–712
31. VALLEE BL, DD ULMER 1972 Biochemical effects of mercury, cadmium, and lead. Annu Rev Biochem 41: 91–128
32. WEIGEL HJ, HJ JÄGER 1980 Different effects of cadmium *in vitro* and *in vivo* on enzyme activities in bean plants (*Phaseolus vulgaris* cv. Sankt Andreas). Z Pflanzenphysiol 97: 103–113
33. YU YB, DO ADAMS, SF YANG 1979 l-Aminocyclopropanecarboxylate synthase, a key enzyme in ethylene biosynthesis. Arch Biochem Biophys 198: 280–286
34. YU YB, DO ADAMS, SF YANG 1980 Inhibition of ethylene production by 2,4-dinitrophenol and high temperature. Plant Physiol 66: 286–290
35. YU YB, SF YANG 1979 Auxin-induced ethylene production and its inhibition by aminoethoxvinylglycine and cobalt ion. Plant Physiol 64: 1074–1077
36. YU YB, SF YANG 1980 Biosynthesis of wound ethylene. Plant Physiol 66: 281–285