Plant Physiol. (1986) 81, 637–641
0032-0889/86/81/0637/05/$0.100/0

The Conversion of 1-(Malonylamino)cyclopropane-1-Carboxylic Acid to 1-Aminocyclopropane-1-Carboxylic Acid in Plant Tissues[1]

Received for publication October 25, 1985 and in revised form February 11, 1986

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ABSTRACT

Since 1-(malonylamino)cyclopropane-1-carboxylic acid (MACC), the major conjugate of 1-aminocyclopropane-1-carboxylic acid (ACC) in plant tissues, is a poor ethylene producer, it is generally thought that MACC is a biologically inactive end product of ACC. In the present study we have shown that the capability of watercress (*Nasturtium officinale* R. Br) stem sections and tobacco (*Nicotiana tabacum* L.) leaf discs to convert exogenously applied MACC to ACC increased with increasing MACC concentrations (0.2–5 millimolar) and duration (4–48 hours) of the treatment. The MACC-induced ethylene production was inhibited by $CoCl_2$ but not by aminoethoxyvinylglycin, suggesting that the ACC formed is derived from the MACC applied, and not from the methionine pathway. This was further confirmed by the observation that radioactive MACC released radioactive ACC and ethylene. A cell-free extract, which catalyzes the conversion of MACC to ACC, was prepared from watercress stems which were preincubated with 1 millimolar MACC for 24 hours. Neither fresh tissues nor aged tissues incubated without external MACC exhibited enzymic activity, confirming the view that the enzyme is induced by MACC. The enzyme had a K_m of 0.45 millimolar for MACC and showed maximal activity at pH 8.0 in the presence of 1 millimolar $MnSO_4$. The present study indicates that high MACC levels in the plant tissue can induce to some extent the capability to convert MACC to ACC.

ACC[2] is the immediate precursor of ethylene, which is synthesized via the following sequence in higher plants: Methionine → SAM → ACC → ethylene (1, 24). In addition to serving as a precursor for ethylene, ACC can also be widely metabolized to the stable conjugate, MACC (3, 6, 7, 11, 13, 16, 19, 22, 23). This conversion is thought to participate in the regulation of ethylene biosynthesis by removing excess ACC (24). Since MACC is a poor ethylene precursor and the conjugation of ACC to MACC is essentially irreversible, MACC is generally thought to be a biologically inactive end product of ACC rather than a storage pool for ACC and hence for ethylene (3, 7, 8, 25). MACC was found to accumulate when high rates of ACC synthesis were induced by water deficit (8) or other factors (3, 6, 20, 23). However, a decrease in MACC content was reported so far only in two systems: in the second node of pea plants (6), and in cocklebur seeds during their late stage of development (20). In the pea plants the decrease in MACC was ascribed to a basipetal transport of MACC to the roots which served as a sink (6), while in cocklebur seeds the fate of MACC is unknown (20). In both systems the decrease in MACC was not attributed to its reuse for ethylene production, since the ethylene produced was derived from the SAM-ACC pathway. In a recent paper, Matern *et al.* (15) stated without accompanying data that an extract of senescent peanut plants contained a particular aminoacylase, which catalyzed the hydrolysis of MACC to ACC. Similary, *N*-malonyl-D-tryptophan has been inferred to liberate D-tryptophan, which is subsequently transformed to IAA, in soybean and tomato cell cultures (17). In the present study we demonstrate the ability of various vegetative tissues to release ACC from exogenously applied MACC, thereby increasing their ethylene production rates.

MATERIALS AND METHODS

Plant Materials. Tobacco plants (*Nicotiana tabacum* L. cv Havana 425) were grown in a greenhouse without supplemental lighting at temperatures between 20 and 30°C. Fully expanded mature leaves (from 11–12 week old plants) were washed under running tap water, surface-sterilized by soaking for 30 s in 0.5% (v/v) NaOCl, and rinsed several times with distilled H_2O), as described previously (2). Watercress plants (*Nasturtium officinale* R. Br.) were purchased from local market and their stems, after thorough washing with distilled H_2O, were used for the experiments. Peanut seeds (*Arachis hypogea* L.), obtained from Lagomarsino Seeds Inc, Sacramento, CA, were planted in vermiculite, and seedlings were grown in a growth chamber at 65% RH and 16 h d (28°C)-8 h night (23°C) cycles as described previously (15). Seedling leaves from various ages were used. In one experiment leaf discs from lettuce (*Lactuca sativa* L.) and soybean (*Glycine max* L.), root segments of radish (*Raphanus sativus* L.) and carrot (*Daucus carota* L.) and fruit plugs of banana (*Musa acuminata* Colla) and chinese gooseberry (*Actinidia chinensis*) were used. All fruits and vegetables were purchased from local market.

Incubation Conditions. Samples of eight tobacco leaf discs weighing about 0.1 g, 10 watercress stem segments weighing about 0.5 g, and 10 discs or segments from each kind of the aforementioned plants, weighing about 0.5 g, were incubated on filter paper in 50-ml Erlenmeyer flasks. All leaf discs were 1 cm in diameter and the root, fruit, or stem segments were 1 cm in diameter and 3 mm thickness. All tissues were incubated in 1 ml water except for tobacco discs, which were incubated in 1 ml of 10 mM Mes buffer, (pH 6.1). Where indicated, various concentrations of MACC (0.2–5 mM), AVG (1 mM), $CoCl_2$ (0.5–2 mM), or labeled *N*-malonyl[2,3-^{14}C]ACC (378 Bq/nmol) were included in the medium. To facilitate MACC uptake, some discs

[1] Supported by a research grant (PCM-8414971) from the National Science Foundation.

[2] Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; AVG, aminoethoxyvinylglycin; MACC, 1-(malonylamino)cyclopropane-1-carboxylic acid; SAM, *S*-adenosylmethionine.

or segments were vacuum infiltrated with H_2O or MACC for 5 min at 0.5 atm prior to their incubation. The flasks were then sealed with rubber serum caps, incubated in darkness at 25°C (or at 30°C for tobacco) and ethylene production, ACC content and MACC content were assayed periodically. Aging of the tissues for 24 h was performed in two manners: tobacco leaf discs were incubated with Mes buffer in darkness at 30°C as described before, while watercress stem segments were floated on 50 ml of aerated water at room temperature.

Determination of Ethylene. A 1-ml gas sample was withdrawn from each flask with a hypodermic syringe at the periods indicated, and the ethylene concentration determined by flame ionization GC using an activated alumina column.

Determination of ACC and MACC. At the indicated periods, samples of 8 tobacco leaf discs or 10 watercress stem segments were extracted twice with 5 ml of boiling 80% ethanol. The ethanol was evaporated under vacuum at 55°C, the residues were dissolved in 2 ml of H_2O, and pigments were removed by addition of 0.5 ml chloroform. ACC content in 0.25 ml aliquots of the aqueous solution was determined according to the method of Lizada and Yang (14). Quantification of MACC in the extract was carried out by hydrolyzing a 0.2 ml aliquot in 2 N HCl at 100°C for 3 h as described previously (9); following neutralization with NaOH, the resulting hydrolysate was assayed for ACC according to Lizada and Yang (14). The difference in ACC content after and before HCl-hydrolysis was taken as the amount of MACC in the extract (8).

Synthesis of Unlabeled and Labeled MACC. Unlabeled MACC was chemically synthesized from ethyl malonylchloride (Aldrich Chemical Co.) and ACC (Calbiochem), according to the method of Satoh and Esashi (19). The purity and identity of MACC was confirmed by paper chromatography, melting point, and GC-MS; ACC assay (14) revealed that it contained 0.05% of free ACC. This amount of contaminated ACC was insignificant to account for the marked increase in ACC levels shown in Figure 2 and Tables II and III. Labeled *N*-malonyl[2,3-^{14}C] ACC ([^{14}C]MACC) was prepared enzymically from malonyl-CoA (Sigma) and [2,3-^{14}C]ACC using a malonyltransferase extracted from etiolated mungbean (*Vigna radiata* L.) hypocotyls as described by Su *et al.* (21).

Radioactive Experiments. To each tobacco leaf disc which was placed abaxial surface up in a small Petri dish with a filter paper, 3 μl of [^{14}C]MACC (3 nmol, 1.13 kBq) were applied and the discs were then vacuum infiltrated for 5 min at 0.5 atm. Samples of eight discs were then incubated abaxial surface down on a filter paper soaked with 1 ml of either 1 mM MACC or 10 mM Mes-buffer (pH 6.1) in sealed 50 ml flasks at 30°C in the dark. Watercress stem segments were fed similarly with 5 μl of [^{14}C] MACC (5 nmol, 1.9 kBq) + 50 μl of 0.5 mM MACC per segment. Samples of two segments were placed in 14-ml tubes, and after vacuum infiltration tubes were sealed and incubated in the dark at 25°C. The radioactive ethylene evolved was absorbed into a plastic center well (Kontes Glass Co.), hung in each flask or test tube, containing a filter paper wick wetted with 0.2 ml of 0.25 M $Hg(ClO_4)_2$ reagent. At the end of the incubation period the paper was soaked in Atomlight scintillation solution and the radioactivity was determined with a Beckman Liquid Scintillation Counter. Similar to the tobacco discs feeding experiments, [^{14}C]MACC + MACC were applied to peanut leaves attached to intact seedlings. After the indicated incubation periods, leaves were rinsed with water and extracted with 80% ethanol as described above. The radioactive metabolites were separated by paper chromatography, using 1-butanol: acetic acid: water (4:1:1.5, v/v) as the solvent system, and after drying the chromatograms were scanned with a Packard radioscanner. Location of unlabeled ACC and MACC standards was visualized by spraying the chromatograms with ninhydrin and pH indicator, respectively. For quantitative determination of [^{14}C]MACC and [^{14}C]ACC the ethanolic extract, after removal of ethanol, was passed through an ion exchange resin column of Dowex-50 (H^+ form) as described previously (12), and the radioactivity of the effluents and eluates was determined individually with a scintillation counter.

Extraction and Assay of MACC-Hydrolase Activity. Watercress stem segments (10 g), preincubated with or without 1 mM MACC for 24 h, were homogenized with 10 ml of 25 mM K-phosphate (pH 7.0). The homogenate was filtered through four layers of cheesecloth and centrifuged at 28,000*g* for 10 min. The supernatant was fractionated with $(NH_4)_2SO_4$; the precipitant obtained at 35 to 80% saturation was redissolved in 1 to 2 ml of 10 mM K-phosphate (pH 7.0) and dialyzed overnight against the same buffer. The dialyzed extract was employed as the enzyme solution. Protein concentration was determined according to Bradford (4). Activity assay was carried out in a total volume of 250 μl with a reaction mixture containing 4 mM MACC, 10 mM K-phosphate (pH 8.0), 1 mM $MnSO_4$, and 0.15 mg protein. After incubation at 37°C for 1 h the reaction was stopped by transferring the tubes to 0°C and the ACC formed was determined according to Lizada and Yang (14). For controls, the assays were similarly carried out with heat-boiled enzymes. The enzyme activity was expressed as nmol ACC formed above the control/mg^{-1} protein$\cdot h^{-1}$. These values were usually 10 times above the controls.

RESULTS

Characterization of the MACC-Induced Ethylene Production. In most plant tissues the ethylene production rate increases as the level of ACC increases. Thus, the MACC-induced ethylene production can be employed as a rough index of ACC release from MACC. While fruit and root segments did not show any increased ethylene production in the presence of exogenous MACC, leaves and stems showed a marked abilibity to convert MACC to ethylene. The highest MACC-induced ethylene production was obtained with watercress stem segments and tobacco leaf discs, showing a 19- and 11-fold stimulation, respectively (Table I), and these two systems were therefore studied in subsequent experiments. The ethylene production rates in the presence of MACC increased after a 4 to 8 h lag period and with aging of the segments in both tobacco and watercress tissues (Fig. 1). The MACC-induced ethylene production of tobacco discs continued to increase during the 28 h of incubation (Fig. 1A), whereas in watercress segments it leveled off after 12 h (Fig. 1B). Aging of the discs or segments for 24 h prior to MACC treatment

Table I. *Effect of Exogenous MACC on Ethylene Production*

Discs or segments excised from various plant tissues were incubated with or without 1 mM MACC and their ethylene production rates during 24 h were measured.

Tissue	Ethylene Production		
	H_2O (A)	MACC (B)	(B)/(A)
	nl g^{-1} 24 h^{-1}		
Leaf			
Lettuce	60	201	3.3
Soybean	125	583	4.6
Tobacco	65	700	11
Stem			
Watercress	9.6	177	19
Root			
Radish	1.2	1.9	1.6
Carrot	12	12	1.0
Fruit			
Banana	103	103	1.0
Chinese gooseberry	211	172	0.8

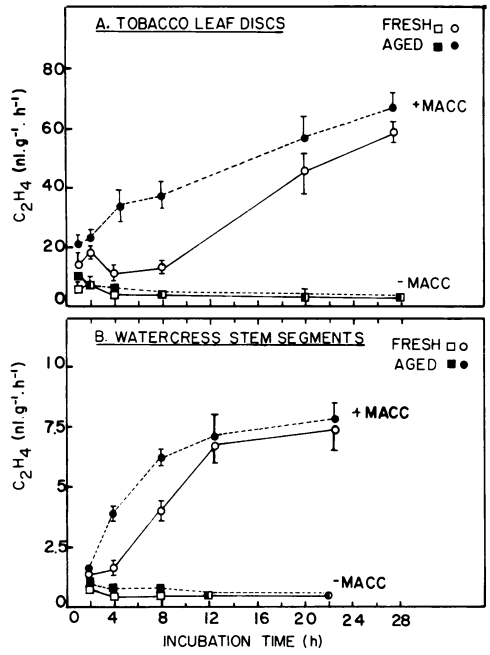

FIG. 1. Time course of MACC-induced ethylene production in fresh and aged tobacco leaf discs (A) and watercress stem segments (B). Fresh and 24 h-aged discs or segments were incubated with or without 1 mM MACC. The bars indicate 2 SE of three replicates.

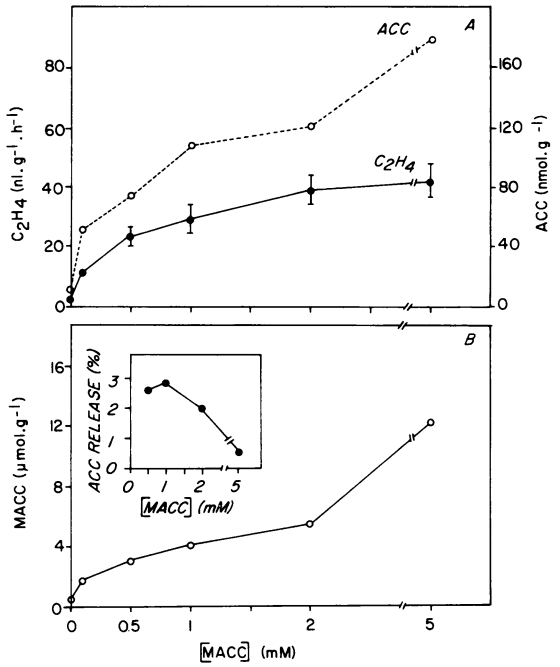

FIG. 2. Effect of MACC concentrations on ethylene production, ACC content (A) and MACC content (B) in tobacco leaf discs. Samples of 8 fresh discs were incubated with the indicated MACC concentrations, and after 8 h their ethylene production rates, ACC and MACC levels were assayed. Inset, degree of MACC conversion to ACC, calculated as the percent of ACC released (A) from MACC taken up (B).

resulted in a 2- to 3-fold stimulation of ethylene production and in shortening the lag period required to get the MACC-induced ethylene response (Fig. 1). This seemed to occur due to facilitated MACC uptake in the aged tissue (data not shown). The data suggest that aging of the segments is necessary to allow the tissue to accumulate a high level of MACC for inducing the hydrolyzing enzyme. This situation may be obtained under physiological conditions during senescence, when considerable amounts of MACC accummulate. By extracting aminoacylase activity, Matern *et al.* (15) have shown that some *N*-malonic acid conjugates, including MACC, are metabolized to a greater extent in extracts of senescent peanut plants than in those of young plants. However, in our *in vivo* system, discs excised from 2-d-old peanut or tobacco leaves and administered with exogenous MACC, showed higher MACC conversion of ACC and ethylene as compared with discs excised from 30-d-old leaves (data not shown).

The MACC-induced ethylene production in tobacco leaf discs increased also with increasing external MACC concentrations (0.2–2 mM), and resulted in increased ACC content (Fig. 2A). While the ethylene production of tobacco discs was saturated at 1 mM MACC, their ACC content further increased with increasing MACC concentrations above 1mM (Fig. 2A). Similar results were obtained also with watercress stem segments (data not shown). The inset of Figure 2B suggests that the maximal percent conversion of MACC to ACC is obtained with 1 mM MACC, and this concentration was employed in most of the experiments.

Metabolism of MACC. The increase of ethylene production in the presence of exogenous MACC results from increased levels of its precursor, ACC (Fig. 2). This may occur via two possible pathways: (a) the MACC applied is metabolized to ACC in the tissue; this pathway should not be sensitive to AVG inhibition, but would be inhibited by Co^{2+}; (b) the MACC applied can promote *de novo* synthesis of ACC from SAM and this pathway will be inhibited AVG. To examine these two possibilities, ethylene biosynthesis inhibitors were employed. Table II demonstrates that the MACC-induced ethylene production was not inhibited by AVG, an inhibitor of SAM to ACC conversion, but was inhibited by $CoCl_2$, and inhibitor of ACC to ethylene (24). These

Table II. *Effect of Ethylene Biosynthesis Inhibitors on MACC-Induced Ethylene Production and ACC Content in Watercress Stem Segments*

Samples of 10 segments were incubated for 24 h with or without 1 mM MACC in the presence of 1 mM AVG or 2 mM $CoCl_2$. Each value represents the mean ± SE of three replicates.

Treatment	Ethylene Production	ACC Content
	nl g^{-1} 24 h^{-1}	*nmol g^{-1}*
H_2O	6.2 ± 1.2	0.9
MACC	182 ± 12	13
MACC + AVG	194 ± 8	16
MACC + Co^{2+}	45 ± 3	35

results eliminate the possibility that MACC stimulates *de novo* ACC synthesis, and suggest that the ACC formed is derived from the MACC applied. The results of Table II also indicate that application of $CoCl_2$, resulted in higher levels of ACC. This could be attributed in part to the inhibitory effect of Co^{2+} on the metabolism of ACC to ethylene and to MACC (16, 21). Direct confirmation of the conclusion that the MACC applied is the source of ACC, was obtained from the observation that radioactive ACC was released from labeled MACC applied to tobacco (Table III) and watercress segments (Fig. 3). When [^{14}C]MACC was applied without unlabeled MACC, about 2% of the radioactive MACC was converted to [^{14}C]ACC (Table III). If the capability to convert MACC to ACC is constitutive, it is expected that the percent conversion of [^{14}C]MACC to ACC should be reduced when 1 mM unlabeled MACC is applied, due to isotope dilution. Nevertheless, the percent conversion of [^{14}C]ACC increased from 2% to more than 7% (Table III). Since the specific radioactivity of ACC found was similar to that of MACC introduced, it is suggested that ACC is derived exclusively from the MACC applied. Figure 3, which represents the metabolism of [^{14}C]MACC in MACC-treated watercress segments, further confirms that free [^{14}C]ACC appeared only after a long incubation

Table III. *Conversion of [^{14}C]MACC to [^{14}C]ACC by Tobacco Leaf Discs*

Samples of 8 discs were incubated in the presence of 40 μl [^{14}C]MACC (24 nmol, 9 KBq) and 1 ml of 0.5 mM $CoCl_2$, with or without unlabeled 1 mM MACC. Labeled and unlabeled MACC and ACC contents of the tissue were assayed after 48 h.

Treatment	MACC/8 discs			ACC/8 discs		
	Bq	*nmol*	*Bq/nmol*	*Bq*	*nmol*	*Bq/nmol*
[^{14}C]MACC	913	7.2	127	18	0.6	30
[^{14}C]MACC + MACC	1067	88	12	77	6.5	12

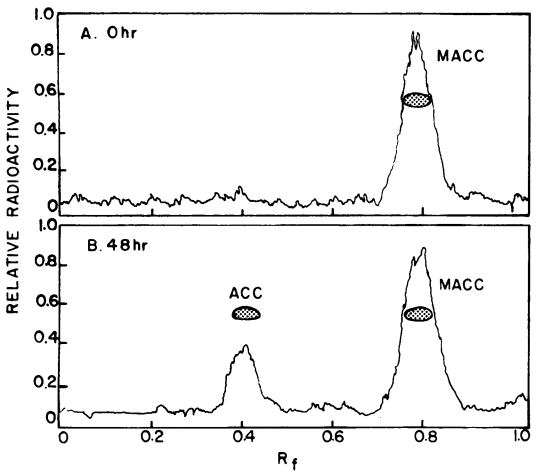

FIG. 3. Metabolism of [^{14}C]MACC in MACC-treated watercress stem segments. Two stem segments (0.1 g) preincubated with 10 μl [^{14}C] MACC (10 nmol, 3.7 kBq) + 100 μl 0.5 mM unlabeled MACC for 0 h (A) or 48 h (B), were extracted with ethanol, chromatographed, and scanned as described in "Materials and Methods." Location of standard ACC and MACC is designated by the circles.

with [^{14}C]MACC + MACC. Similar results were obtained also with tobacco leaf discs, suggesting that the process of MACC hydrolysis is activated by an inducible enzyme.

In the present experiments, plant tissues were incubated for a relatively long time. Thus, there is a possibility that microorganisms grown in the aged plant tissues are directly or indirectly involved in the conversion of MACC to ACC. This question was examined by conducting the incubation under sterile conditions. The degree of MACC conversion to ACC in those experiments was not significantly lower than in the nonsterile experiments (data not shown). Also, application of several antibiotics and fungicides (Gentamycin Sulfate, Penicillin, Streptomycin, and Fungizone) did not significantly reduce the MACC-induced ethylene production. It is therefore unlikely that the high levels of ACC and ethylene produced are caused by bacterial contamination.

Activity of MACC-Hydrolase. A cell-free extract capable of catalyzing the conversion of MACC to ACC was prepared from watercress stems. Attempts to extract the corresponding enzyme from tobacco leaves were unsuccessful. Extracts of fresh watercress stems showed low enzymic activity, which could be increased by 3-fold when the stems had been aged for 24 h (Table IV). However, the highest enzymic activity was obtained with extracts prepared from segments which were preincubated with 1 mM MACC for 24 h (Table IV). This confirms our previous data showing that the enzyme is induced by high levels of external MACC. The amount of ACC released was linear up to 3 h and for routine assays a 1-h incubation was chosen. The temperature optimum of the reaction was found to be 50°C (data not shown),

Table IV. *Effect of Preincubation with MACC on the Development of MACC-Hydrolase Activity in Extracts of Watercress Stem Segments*

Enzyme was extracted from fresh or aged stem segments (10 g), preincubated with or without 1 mM MACC for 24 h, and assayed as described in "Materials and Methods."

Treatment	MACC Hydrolysis Activity
	nmol ACC mg^{-1} protein h^{-1}
None	0.3
24 h Aging	0.8
24 h Aging + MACC	10.6

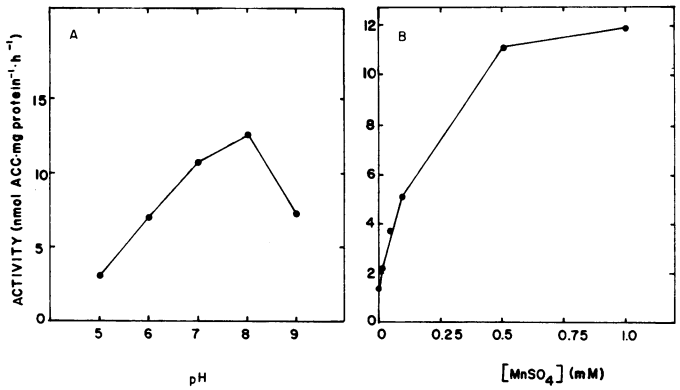

FIG. 4. Effect of pH(A) and $MnSO_4$ concentrations (B) on MACC-hydrolase activity. Enzyme was extracted from watercress stem segments, which had been preincubated with 1 mM MACC for 24 h, and its activity was assayed as described under "Materials and Methods" except that the pH (A) and $MnSO_4$ concentrations (B) were varied where indicated.

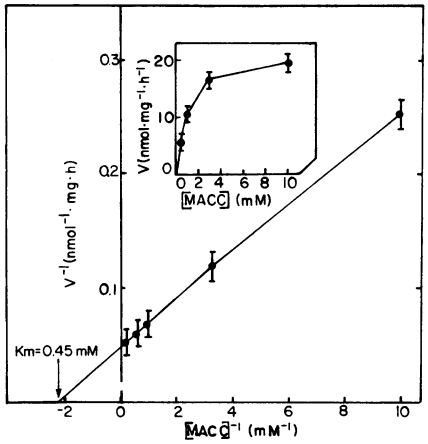

FIG. 5. Lineweaver-Burk plot for MACC-hydrolase activity. Watercress enzyme was assayed at pH 8.0 as detailed in Figure 4 with various MACC concentrations.

but routine assays were performed at 37°C with a reaction rate of 87% of the maximum. The enzyme had a pH optimum at 8 (Fig. 4A) in 10 mM K-phosphate, and its catalytic activity was enhanced by $MnSO_4$, achieving saturation at 1 mM (Fig. 4B). Mg^{2+} and Zn^{2+} ions gave only 70% of the enzymic activity obtained with Mn^{2+}, while Cu^{2+}, Hg^{2+} or Co^{2+} caused inhibition (data not shown). The MACC-hydrolase activity exhibited normal Michaelis-Menten kinetics when the substrate concentration was varied (Fig. 5, inset). The double-reciprocal Lineweaver-Burk plot of velocity with variable MACC concentrations (Fig. 5) allowed the determination of an apparent K_m of 0.45 mM for MACC. This high K_m value further indicates the relatively high levels of MACC required for the MACC-hydrolase.

DISCUSSION

N-Malonyl conjugates of D-amino acids have been isolated from various higher plants, either as natural constituents or after feeding with D-amino acids (18). Recent interest has been focused on MACC, the malonyl conjugate of ACC. It was demonstrated that most higher plant tissues were capable of metabolizing both endogenous and exogenous ACC to MACC (3, 7, 9–11, 13, 16, 19, 20, 22, 23) and the enzyme responsible for this process is constitutive and widespread (3, 24). So far, MACC was thought to be a metabolically inactive compound which does not serve to a significant extent as a storage pool for ACC and hence for ethylene production (24). In the present study we have further examined the fate and possible metabolism of MACC after feeding leaf and stem segments with exogenous MACC, and we have established conditions under which MACC can serve as a source for ACC and ethylene. While exogenously applied MACC did not promote ethylene production in peanut (8) and cocklebur (20) seeds, and in root and fruit tissues (Table I), we have shown that watercress stem sections and tobacco leaf discs exhibited a marked MACC-induced ethylene production (Table I). Similarly, whole peanut leaves attached to the plant seedlings could convert MACC to ACC (data not shown).

Unlike previous reports (3, 24), our results indicate that under certain conditions MACC can be hydrolyzed back to ACC in some tissues. The conditions required to obtain a significant MACC hydrolysis from vegetative tissues are summarized as follows: (a) long incubation time (several h); (b) high MACC concentration for inducing the enzyme; and (c) high MACC concentration for the enzyme activity. There are at least three possible explanations for the lack of or low MACC conversion to ACC reported in some tissues. First, the MACC-hydrolase activity can be induced only when MACC content in the tissue is high enough. Once the enzyme is induced, it still requires high levels of MACC for activity, as indicated by its high K_m value (0.45 mM). Endogenous MACC levels reported so far in various plant tissues (during short incubations) ranged from 24 to 187 nmol/g, either after stress or ACC feeding (3, 6–8, 11–13, 16, 20, 23), and these values are much below the K_m of the enzyme. This may explain why an insignificant MACC hydrolysis could be detected in these systems. Second, the uptake rate of MACC by the plant tissues is much slower than that of ACC. Thus, longer incubation periods are needed to exhibit a significant MACC hydrolase activity. Third, MACC accumulated endogenously may be highly compartmentalized in some tissues and therefore become unavailable for the hydrolyase enzyme.

Since a significant conversion of MACC to ACC could be induced only by external MACC, and since the endogenous MACC levels are normally far below the apparent K_m for MACC hydrolase, the physiological importance of this process is obscure. However, the present study implies that even though very little metabolism of MACC probably occurs under physiologcial conditions, the potential exists for MACC to serve as a source of ACC and ethylene when its level increases. The existence of the capacity to hydrolyze certain malonyl conjugates is exemplified by the observations that *N*-malonyl-D-tryptophan exhibits an IAA-like activity, presumably though its hydrolysis to D-tryptophan which serves as an IAA precursor (17).

The cell-free extract prepared from MACC-treated watercress stems catalyzes the cleavage of the amide bond of MACC to form ACC and presumbably malonic acid, and such an activity can be classified as an aminoacylase. Preliminary characterization indicates that it is a metal-requiring enzyme, whose activity, like other aminopeptidases (5), is enhanced by Mn^{2+} ions (Fig. 4B). Since ACC-malonyltransferase isolated from plant tissues also catalyzes the malonylation of D-amino acids (12, 21), it is of interest to know whether the MACC-hydrolase is also capable of hydrolyzing *N*-malonyl-D-amino acids.

Acknowledgments—We wish to thank Dr. J. E. Devay from the Department of Plant Pathology, University of California, Davis for providing the tobacco plants, and Ms. Betty Rumbaugh for drawing the figures.

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