Plant Physiol. (1988) 88, 109–114
0032-0889/88/88/0109/06/$01.00/0

S-Adenosylmethionine-Dependent Inactivation and Radiolabeling of 1-Aminocyclopropane-1-Carboxylate Synthase Isolated from Tomato Fruits[1]

Received for publication December 29, 1987 and in revised form April 1, 1988

SHIGERU SATOH[2] AND SHANG FA YANG*
Mann Laboratory, Department of Vegetable Crops, University of California, Davis, California 95616

ABSTRACT

1-Aminocyclopropane-1-carboxylic acid (ACC) synthase was partially purified from the homogenate of wounded tomato (*Lycopersicon esculentum* Mill.) pericarp tissue by $(NH_4)_2SO_4$ fractionation followed by conventional column chromatography with diethylaminoethyl-Sepharose, Sephadex G-150, Affi-Gel blue and hydroxylapatite. The partially purified ACC synthase preparation attained a specific activity of about 12,000 nmoles per hour per milligram protein. Employing this enzyme preparation, we confirmed that the ACC synthase was inactivated by its substrate, *S*-adenosyl-L-methionine (SAM), during its catalytic action. When the partially purified enzyme preparation was incubated with [3,4-^{14}C]SAM and the resulting proteins were analyzed by sodium dodecyl sulfate-gel electrophoresis, only one radioactive protein band was observed. This protein was thought to be ACC synthase based on its molecular mass of 50 kD and on the fact that it was specifically bound to a monoclonal antibody against ACC synthase (AB Bleecker *et al.* 1986 Proc Natl Acad Sci USA 83, 7755–7759). These results suggest that the substrate SAM acts as an enzyme-activated inactivator of ACC synthase by covalently linking a fragment of SAM molecule to the active site of ACC synthase, resulting in the inactivation of the enzyme.

Ethylene is a plant hormone regulating many aspects of plant growth and development, such as seed germination, seedling elongation, fruit ripening, and tissue senescence (1, 12). It has been established that ethylene is biosynthesized from methionine via SAM[3] and ACC and that the rate-limiting step in ethylene biosynthesis is the conversion of SAM to ACC, catalyzed by ACC synthase (21). Since ACC synthase is rapidly turned over in plant tissues, the concentration of the enzyme in plant tissue is determined not only by the synthesis of the enzyme but also by its inactivation (8, 20).

Recently, Satoh and Esashi (18) reported that ACC synthase obtained from mung bean hypocotyls is inactivated by its substrate, SAM, during its catalytic action. Similar finding was independently reported in a meeting abstract by Boller (3) with ACC synthase isolated from tomato pericarp tissue. Since the half-life of ACC synthase *in vivo* was similar to that *in vitro*, Satoh and Esashi (18) have suggested that the SAM-dependent inactivation of ACC synthase is responsible for the rapid decrease of the enzyme activity found in intact tissues. Since SAM probably acts as an enzyme-activated irreversible inactivator in addition to serving as a substrate for the enzyme, we suggest that the inactivation of ACC synthase by SAM results from a covalent linkage of a fragment of SAM molecule into the active site of ACC synthase.

In this communication we report that the partially purified ACC synthase preparation from tomato pericarp was radiolabeled by treatment with radioactive SAM labeled at C-3,4 of the methionine moiety. We present evidence showing that there is only one radiolabeled protein and this protein is ACC synthase.

MATERIALS AND METHODS

Plant Material. Tomato (*Lycopersicon esculentum* Mill.) fruits at the pink stage of ripeness were bought at a local market. The fruits were cut into 5-mm thick slices, and locular material was removed and discarded. The remaining pericarp slices were placed on moist paper towel and incubated for 18-h at room temperature in the dark. The pericarp tissue, weighing 6.08 kg, was divided into 15 batches and stored at −80°C until homogenization.

ACC Synthase Assay. ACC synthase activity was measured by incubating an enzyme sample (5–20 μL) at 30°C for 15 min with 200 μM SAM, 3.1 μM PLP, and 75 mM Hepes-KOH buffer (pH 8.2) in a total volume of 0.4 mL. The amount of ACC formed was determined by the method of Lizada and Yang (13). One unit of enzyme was defined as that which converts 1 nmol of SAM to ACC per h at 30°C.

Enzyme Extraction and Purification. The extraction and purification of ACC synthase were carried out at 4°C. All buffer solutions, except that used for homogenization, contained 5 μM PLP, 0.5 mM 2-ME, 0.1 mM phenylmethylsulfonyl fluoride, and 10% (w/v) glycerol. To a 400-g batch of partially thawed tomato pericarp tissue was added 400 mL of 100 mM K-phosphate, pH 8.0, containing 5 μM PLP, 4 mM 2-ME, 1 mM EDTA, and 0.1 mM phenylmethylsulfonyl fluoride. The tissue was homogenized in a Waring blender for 1 min at maximum speed. The homogenate was squeezed through four layers of cheese cloth, and a filtrate of 500 to 550 mL was recovered. The combined filtrates (7.9 L) from 15 batches of tomato pericarp tissue were adjusted to pH 8.0 with 4 M KOH. Solid $(NH_4)_2SO_4$ was added in portions to the filtrate to make 40% saturation with stirring over a 2-h period, during which the solution was maintained at pH 7.5 to 8.0 with 4 M KOH. After standing overnight, the resulting suspension was centrifuged at 18,000*g* for 15 min, and the precipitate was discarded. To the supernatant obtained (8.68 L) was added solid $(NH_4)_2SO_4$ to make 75% saturation. After stand-

[1] This work was supported by grant DMB-8414971 from the National Science Foundation.

[2] Permanent address: Department of Biological Science, Tohoku University, Kawauchi, Sendai 980, Japan.

[3] Abbreviations: SAM, *S*-adenosyl-L-methionine; ACC, 1-aminocyclopropane-1-carboxylic acid; AVG, aminoethoxyvinylglycine; 2-ME, 2-mercaptoethanol; PLP, pyridoxal phosphate.

ing overnight, the precipitate formed was recovered by centrifugation and was dissolved in 220 ml of 33 mM K-phosphate, pH 8.0. The solution was dialyzed for 3 d against two changes of 6 L of the same buffer, and any insoluble material was removed by centrifugation at 18,000*g* for 15 min.

The enzyme solution (223 ml) was applied to a DEAE-Sepharose column (4.5 cm × 6 cm, bed volume 95 mL), which had been equilibrated with 33 mM K-phosphate, pH 8.0. After washing with 400 mL of the column buffer, the adsorbed proteins were eluted from the column with 400 mL of a 0 to 300 mM linear gradient of KCl containing the column buffer at a flow rate of 1 mL min^{-1}. Fractions of 8 mL were collected and monitored for protein (A_{280}) and ACC synthase activity. Active fractions were combined and concentrated to about 28 ml by dialyzing against polyethylene glycol-8000. The condensed enzyme solution was loaded on a Sephadex G-150 column (5 cm × 15 cm, bed volume 1080 mL), which had been equilibrated with 33 mM K-phosphate, pH 8.0. Proteins were eluted from the column with 1 L of the column buffer at a flow rate of 1.5 mL min^{-1}. Fractions of 8 mL were collected and assayed for protein (A_{280}) and the enzyme activity. The active fractions were combined and condensed to about 50 mL by dialyzing against polyethylene glycol-8000.

The condensed enzyme solution (50 mL) was dialyzed for 2 d against two changes of 1.6 L of 10 mM K-phosphate, pH 6.8. The dialysate (68 mL) was applied to an Affi-Gel blue column (1 cm × 3.8 cm, bed volume 3 mL), which had been equilibrated with 10 mM K-phosphate, pH 6.8 (column buffer). The column was washed with 15 mL of the column buffer, and elution was carried out with a linear gradient established between 20 mL of the column buffer and 20 mL of 100 mM K-phosphate, pH 8.0, at a flow rate of 0.33 mL min^{-1}. Fractions of 2 mL were collected and assayed for the enzyme activity, and those with higher specific enzyme activity were combined to make 16 mL. The combined enzyme solution was condensed to 3 mL using Centricon-30 (Amicon) and was taken up in 13 mL of 10 mM K-phosphate (pH 8.0). This procedure was repeated, and the final enzyme solution was taken up in 10 mL of the above buffer.

The above enzyme solution was loaded on a hydroxylapatite column (0.7 cm × 3 cm, bed volume 1.15 mL), which had been equilibrated with 10 mM K-phosphate, pH 8.0 (column buffer). After washing with 5 mL of the column buffer, proteins were eluted from the column with 24 mL of a linear gradient between 10 and 300 mM K-phosphate at a flow rate of 0.2 mL min^{-1}. Fractions of 0.7 mL were collected and those having higher specific enzyme activity were combined. This enzyme preparation is referred to as the partially purified enzyme preparation and was stored at −80°C until use.

Protein contents were determined according to Bradford (5) with BSA as a standard.

Assays for Inactivation of ACC Synthase. A sample of the partially purified enzyme (11.6 units) was incubated with 200 μM SAM, 3.75 μM PLP, 1 mg BSA, and 75 mM Hepes-KOH (pH 8.2) in a total volume of 0.96 mL at 30°C. After incubation for various periods, a 100 μL portion of the reaction mixture was withdrawn, cooled in an ice-bath, and passed through a small column of Sephadex G-25 (bed volume 1 ml), which had been equilibrated with 33 mM K-phosphate (pH 8.0) containing 5 μM PLP and 0.5 mM 2-ME. The eluate containing protein was collected and assayed for ACC synthase as described above.

Immunoaffinity Purification of ACC Synthase. Immunoaffinity purification of ACC synthase from the partially purified enzyme preparation was carried out as described by Bleecker *et al.* (2). The immunoaffinity gel was a gift from A. B. Bleecker and H. Kende and was prepared by conjugating the ACC synthase monoclonal antibody to Sepharose-4B (2). 2-ME was added to the extract eluted from the immunoaffinity gel to make a final concentration of 5% (v/v) before electrophoresis.

Gel Electrophoresis and Fluorography. SDS-PAGE was conducted using 10% gels by the methods of Laemmli (11). Gels were stained with Coomassie blue and prepared for fluorography by soaking in Amplify (Amersham). After drying, they were exposed to Kodak XAR-5 x-ray film at −80°C for 1 to 14 d.

Radiolabeling of ACC Synthase. A sample of the partially purified enzyme (340 μg, 3858 units) was incubated for 6 h at 30°C with 186 μM [3,4-^{14}C]SAM (11.8 μCi), 3.6 μM PLP, and 120 mM Hepes-KOH (pH 8.2) in a total volume of 1.28 mL. After incubation, the reaction mixture was passed through a Sephadex G-25 PD-10 column, and the eluate containing protein was collected. The protein fraction was washed twice with 2 mL of 10 mM K-phosphate (pH 8.0) and condensed to a small volume using Centricon-30. The protein was analyzed by SDS-PAGE before and after immunoaffinity purification as described above.

Reagents. SAM *p*-toluenesulfonate salt (Lot No. 126F-7130) was bought from Sigma Chemical Co. [3,4-^{14}C]SAM was from Research Products International.

RESULTS

Purification of ACC Synthase. Ripe tomato fruit tissue is known to be a good source of ACC synthase and its activity can be further increased by wounding (4, 8, 22, 23). Observations from this laboratory (T McKeon, M Nieder, unpublished results) indicated that slicing tomato fruit into 5-mm thick sections, followed by incubation overnight, was effective in inducing ACC synthase activity. In this study, we extracted ACC synthase from such sliced and incubated tomato fruits tissue.

ACC synthase was purified 138-fold, with 17% recovery, from the homogenate of 6.08-kg tomato pericarp tissue following conventional $(NH_4)_2SO_4$ fractionation and column chromatography with DEAE-Sepharose, Sephadex G-150, Affi-Gel blue and hydroxylapatite (Table I). A specific enzyme activity of 12,000 units (mg protein)$^{-1}$ was achieved, and this value was similar to that reported by Bleecker *et al.* (2). SDS-PAGE of the partially purified enzyme revealed many dye-stained bands, but only four bands were seen when the enzyme preparation was purified by the immunoaffinity gel. It was evident that three of the four bands originated from the immunoaffinity gel (Fig. 1, lane C). Bleecker *et al.* (2) have shown that these bands were IgG subunit proteins. Thus, the remaining protein band was thought to be ACC synthase, as its M_r was 50 kD, coinciding with that previously reported for tomato ACC synthase (2, 16).

Characterization of ACC Synthase. When enzyme activity was assayed in 200 mM K-phosphate at different pH between 6.5 and 10.5, the maximum ACC production occurred at pH 9 (Fig. 2). As the pH of the reaction mixture increased from 7.5 to 9.5, K_m for SAM increased from 38 to 167 μM, but K_i for AVG, a competitive inhibitor (4, 21), decreased from 2.9 to 0.25 μM (Fig. 3). These findings indicate that pH changes exerted different effects on the affinity of ACC synthase for its substrate, SAM, and for its competitive inhibitor, AVG. The K_m for SAM was 32.3 μM at pH 8.0. This value was slightly higher than those of 13 to 27 μM reported previously (4, 15, 22). Khani-Oskouee *et al.* (9, 10) reported that the K_m of tomato ACC synthase was 15 ± 5 μM with (−)-SAM and 40 ± 10 μM with (±)-SAM. Since commercial SAM preparations contain varied amounts of inactive (+)-SAM and other impurities (6), accurate estimates of K_m for SAM is difficult. Since K_m values increased at pH above 8.5 (Fig. 3), we chose to carry out ACC synthase assay at pH 8.2 in the subsequent experiments.

SAM-Dependent Inactivation of ACC Synthase. SAM-dependent inactivation of tomato ACC synthase was confirmed using this partially purified enzyme preparation. At various incubation times, protein fractions were separated from the reaction mixture

Table I. *Partial Purification of ACC Synthase from the Homogenate of 6.08-kg Tomato Pericarp Tissue*

Fraction	Total Activity	Protein	Specific activity	Purification	Recovery
	units	*mg*	*units·mg*$^{-1}$	*-fold*	*%*
40–75% $(NH_4)_2SO_4$	132,676	1,561.0	85.0	1.0	100.0
DEAE-Sepharose	64,434	317.6	202.9	2.4	48.6
Sephadex G-150	51,167	81.6	627.7	7.4	38.6
Affi-Gel blue	19,203	5.99	3,205	37.7	14.5
Hydroxylapatite	22,338	1.90	11,732	138	16.8

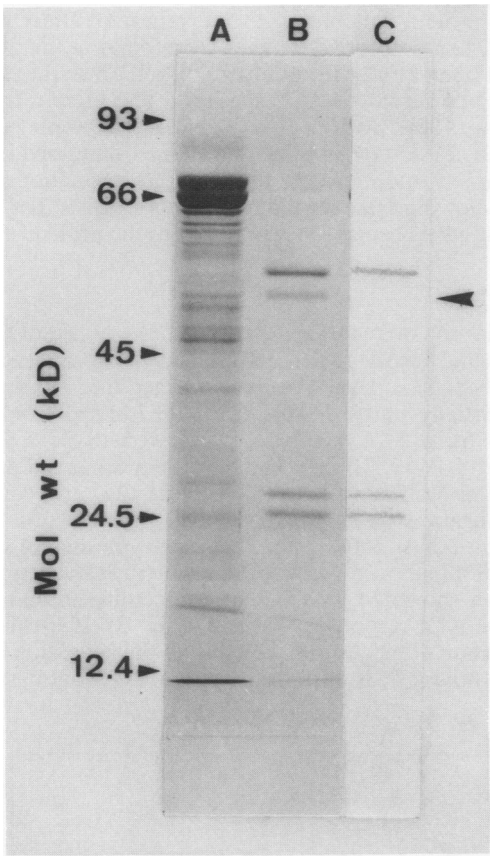

FIG. 1. SDS-PAGE profiles of ACC synthase preparations stained with Coomassie blue. Lanes: A, a sample (102 μg protein) of the partially purified enzyme preparation after hydroxylapatite column purification; B, proteins eluted from the immunoaffinity gel after incubation of the gel with the enzyme preparation (340 μg protein) used in lane A; C, proteins eluted from the immunoaffinity gel, which had not been incubated with the enzyme preparation. The M_r standards: 93 kD, phosphorylase *b*; 66 kD, BSA; 45 kD, ovalbumin; 24.5 kD, α-chymotrypsinogen; 12.4 kD, cytochrome *c*.

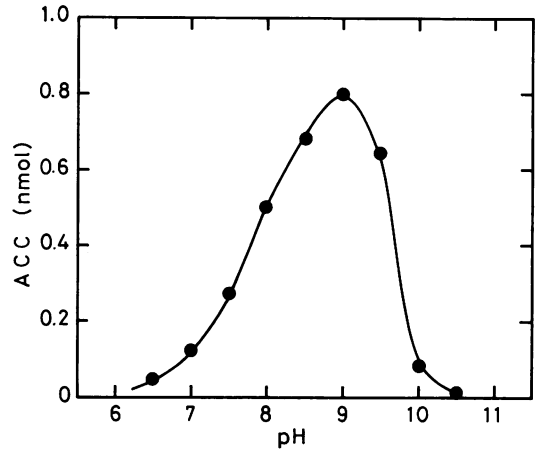

FIG. 2. pH dependence of ACC synthase activity. A sample of the partially purified ACC synthase (2.7 units) was incubated with 200 μM SAM and 5 μM PLP in 200 mM K-phosphate at various pH as specified for 15 min at 30°C.

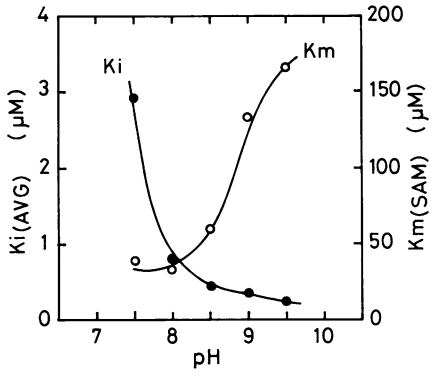

FIG. 3. Effect of pH on K_m for SAM and K_i for AVG. ACC synthase activities were measured as described in Figure 3, with SAM concentration varying from 20 to 100 μM and AVG concentration varying from 0 to 10 μM. K_m and K_i were obtained from Lineweaver-Burk plots.

by gel filtration and assayed for enzyme activity. Figure 4 shows the changes of the remaining ACC synthase activity on a log scale as a function of incubation time. The straight line relationship indicates the enzyme inactivation follows a first-order kinetics with a half-life of 66 min in the presence of 200 μM SAM. Little enzyme inactivation was observed without SAM.

Previous results showed that AVG, a competitive inhibitor with respect to SAM for ACC synthase (4, 21), inhibited the enzyme from being inactivated by SAM (18). This observation was reconfirmed in this study using tomato ACC synthase. Figure 5A shows the time courses of ACC production with or without AVG when the initial concentration of SAM was 100 μM. The rate of ACC production gradually decreased with time. We defined the remaining enzyme activity at a given time during the incubation period as the amount of ACC produced during the subsequent 15-min period. A plot of remaining enzyme activity in log scale as a function of time gave a straight line, indicating the time-dependent inactivation of the enzyme activity (Fig. 5B). Addition of AVG to the reaction mixture decreased ACC production but suppressed the inactivation of ACC synthase activity. This is illustrated by the results that the half-life of the enzyme increased from 54 min in the absence of AVG to 108 min with 2.5 μM AVG (Fig. 5B). These findings agreed well with those obtained previously with ACC synthase from mungbean hypocotyls (18) and further suggest that SAM inactivates ACC synthase during the catalytic action of the enzyme.

***In Vitro* Labeling of ACC Synthase with [^{14}C]SAM.** If SAM

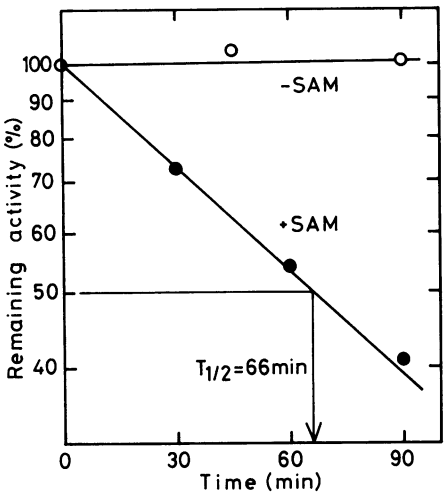

FIG. 4. Dependence of remaining ACC synthase activity on incubation time. A sample of the partially purified ACC synthase (11.6 units) was incubated at 30°C with or without 200 μM SAM and aliquots of the reaction mixture were withdrawn at specified time and subjected to gel filtration to separate protein. The remaining ACC synthase activity in the protein fraction was measured by the standard assay method.

acts as an enzyme-activated irreversible inactivator for ACC synthase, we hypothesized that a part of SAM molecule may bind covalently to the active site of the enzyme, resulting in its inactivation. To test this hypothesis, we incubated the partially purified enzyme with [3,4-^{14}C]SAM in the presence of PLP for 6 h at 30°C. Since this incubation period is equivalent to 5 to 6 half-lives of the enzyme (Figs. 4 and 5), it is expected that inactivation of the enzyme by SAM (or the putative incorporation of radioactivity into the enzyme) should be essentially complete. Proteins were separated from the reaction mixture and analyzed by SDS-PAGE both before and after immunoaffinity purification. To assure that there was enough radioactive protein, we loaded with a larger amount of protein in the experiment of Figure 6 than that of Figure 1. It is to be noted that the SDS-PAGE of the protein fraction purified with immunoaffinity gel shows some bands other than that of 50 kD on the dye-stained gel, especially, that of 66 kD (Fig. 6, lane B). These proteins were not seen on the dye-stained gel in Fig. 1. It is possible that these proteins were not bound specifically to the immunoaffinity gel but were not completely removed prior to elution from the immunoaffinity gel. Since these bands were not radiolabeled, further characterization of these bands was not attempted.

Fluorography shows that only one protein at 50 kD was labeled by treatment with the [^{14}C]SAM (Fig. 6, lane C) and this radiolabeled protein was bound to the immunoaffinity gel (Fig. 6, lane D). No radioactive proteins were found in the fraction that was not bound to the immunoaffinity gel. Thus, the radiolabeled protein appears to be ACC synthase. The protein fraction was heated at 100°C with 5% (w/v) 2-ME, electrophoresed in the presence of 0.1% (w/v) SDS, and stained/destained in an acidic methanol solution prior to fluorography. Retention of radioactivity following these drastic treatments suggests that radioactivity must have been covalently linked to the protein.

DISCUSSION

Previously, we reported (18) that ACC synthase obtained from mungbean hypcotyls was inactivated by its substrate SAM during its catalysis. The present study confirmed that this was also true with partially purified ACC synthase isolated from wounded tomato fruit. ACC synthase is believed to be a PLP-linked enzyme (4, 7, 19, 21, 22). The reaction mechanism proposed by Yang and Hoffman (21) and Yu *et al.* (22) and confirmed by Ramalingam *et al.* (17) involves the formation of a Schiff base (aldimine bond), between the PLP-coenzyme and SAM, followed by a γ-elimination yielding ACC and methylthioadenosine. AVG has been shown to be a potent competitive inhibitor of ACC synthase with respect to SAM (4) (Fig. 3). If SAM-dependent inactivation of ACC synthase involves the activation of SAM by ACC synthase, it is anticipated that AVG would suppress this

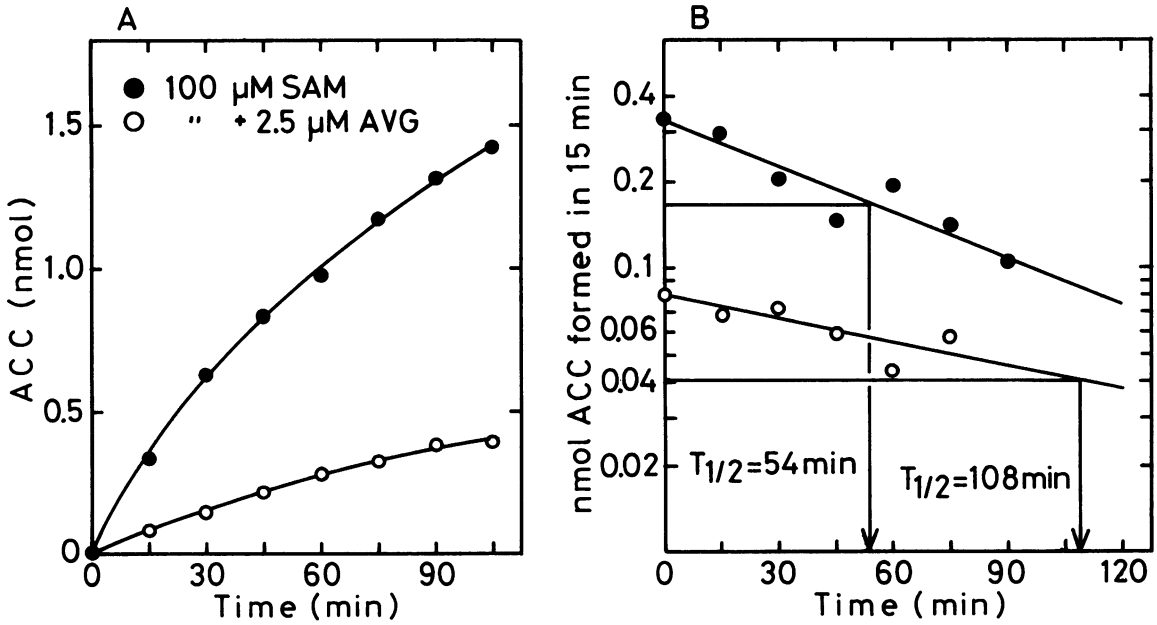

FIG. 5. Time courses of ACC production (A) and change in the remaining ACC synthase activity during incubation (B). A, Samples of the partially purified ACC synthase (2.4 units) were incubated with 100 μM SAM, 3.1 μM PLP, and 75 mM Hepes-KOH (pH 8.2) in the absence or presence of 2.5 μM AVG at 30°C. At 15-min intervals, an aliquot was withdrawn and assayed for ACC content. B, The remaining activity at a given incubation time was calculated from the data of (A) as the amount of ACC formed during a subsequent 15-min incubation period, and was plotted on a log scale. The half-life values ($T_{1/2}$) of ACC synthase were estimated from the straight lines.

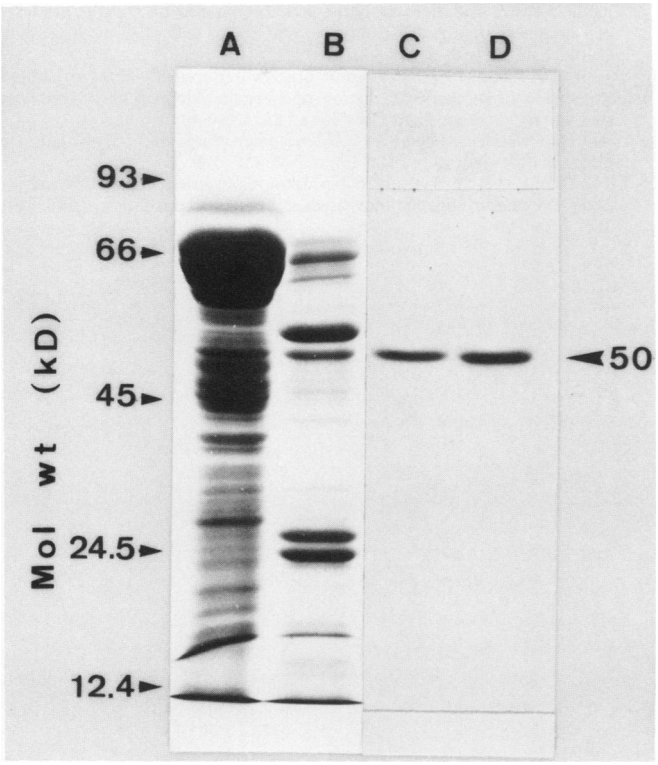

FIG. 6. SDS-PAGE analysis of [^{14}C]SAM-treated ACC synthase preparations. Lanes A and B are Coomassie blue stain, and lanes C and D are the fluorograph of lane A and B, respectively, which were exposed to film for 1 d. A and C, A sample of partially purified enzyme preparation (340 μg) which has been incubated with [^{14}C]SAM. B and D, Proteins eluted from the immunoaffinity gel after incubating the gel with 510 μg of the [^{14}C]SAM-treated enzyme preparation used in lanes A and C. In a prior experiment, we had determined that when 340 μg of this enzyme preparation was incubated with 200 μl of the immunoaffinity gel, more than 90% of the enzyme activity was removed by the gel.

SAM-dependent inactivation of ACC synthase. This was found to be true (18) (Fig. 5). Thus, it is reasonable to propose that the inactivation process involves the formation of ACC synthase-SAM complex, which is then transformed into an intermediate; since this intermediate is so reactive that a fragment of SAM molecule may covalently link to the active site of ACC synthase, leading to the irreversible inactivation of ACC synthase. Most PLP-linked enzymes are known to have a lysine residue in their active site, where the ϵ-amino group of lysine makes a Schiff base with PLP coenzyme. Assuming that this is also true with ACC synthase, it is expected that when SAM displaces the lysine residue of ACC synthase and forms the new Schiff base, this ϵ-amino group of lysine residue at active site becomes free. The above hypothesis predicts that a fragment of SAM is incorporated into ACC synthase at its active site, leading to inactivation of ACC synthase. To test this hypothesis, we treated the partially purified enzyme with [3,4-^{14}C]SAM in the presence of PLP, and analyzed the treated sample by SDS-PAGE. Fluorography shows that only one protein was radiolabeled (Fig. 6). The protein was judged to be ACC synthase based on its mol wt and its affinity for the immunoaffinity gel. It is to be noted that such an inactivated ACC synthase is also recognized by the monoclonal antibodies. It is possible that the inactivation of ACC synthase by SAM is the first step in its turnover, which is followed by a preferential proteolytic degradation of the inactivated enzyme.

It is well known that $NaBH_4$ inactivates many PLP-utilizing enzymes by reducing the aldimine linkage between PLP and the ϵ-amino group of lysine residue of enzymes, and the employment of $NaB[^3H]_4$ results in radiolabeling of enzymes (14). Recently, Privalle and Graham (16) showed that tomato ACC synthase was radiolabeled upon treatment with $NaB[^3H]_4$ in the presence of PLP. We confirmed that 0.2 mM $NaBH_4$ inactivates more than 95% of ACC synthase in our partially purified enzyme preparation. When our enzyme preparation was similarly incubated with $NaB[^3H]_4$ at pH 8 with 5 μM PLP, many proteins, including the 50-kD protein, were radiolabeled, indicating that the reaction was not specific for ACC synthase. However, when the labeled enzyme preparation was purified by the immunoaffinity gel before electrophoresis and fluorography, only one radiolabeled band was detected, which exhibited a M_r of 50 kD (our unpublished results). These results demonstrate again the specific affinity of the immunoaffinity gel.

In the present study, we have shown that C-3,4 of methionine moiety in SAM is linked to ACC synthase during the autoinactivation process. However, it is yet to be determined what part of SAM, beside C-3,4 of methionine moiety, is incorporated into ACC synthase. This can be determined by examining the radiolabeling of ACC synthase with radioactive SAM labeled at different positions, and by analyzing the amino acid residue(s) of ACC synthase to which the radioactive SAM moiety links. It is well known that the natural (−)-SAM can be converted into (+)-SAM. Although (+)-SAM does not serve as the substrate for many SAM-utilizing enzymes, including ACC synthase (9, 10), (+)-SAM is capable of binding to ACC synthase and acts as an inhibitor for the conversion of (−)-SAM to ACC (9). We do not know whether one or both diastereoisomers are responsible for this SAM-dependent inactivation of ACC synthase. These questions are currently under investigation.

Acknowledgments—We are very grateful to Drs. A. B. Bleecker and Hans Kende (Michigan State University) for providing us the immunoaffinity gel and to Dr. D. Della Penna for the help in preparation of fluorograph.

LITERATURE CITED

1. ABELES FB 1973 Ethylene in Plant Biology. Academic Press, New York
2. BLEECKER AB, WH KENYON, SC SOMERVILLE, H KENDE 1986 Use of monoclonal antibodies in the purification and characterization of 1-aminocyclopropane-1-carboxylate synthase, an enzyme in ethylene biosynthesis. Proc Natl Acad Sci USA 83: 7755–7759
3. BOLLER T 1985 Time-dependent inactivation of 1-aminocyclopropane-1-carboxylate synthase by its substrate, *S*-adenosylmethionine. *In* The 12th International Conference on Plant Growth Substances, Abstract. Heidelberg, p 36
4. BOLLER T, RC HERNER, H KENDE 1979 Assay for an enzymatic formation of an ethylene precursor, 1-aminocyclopropane-1-carboxylic acid. Planta 145: 293–303
5. BRADFORD MM 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248–254
6. CREASON GL, JT MADISON, JF THOMPSON 1985 Soybeans and radish leaves contain only one of the sulfonium diastereoisomers of *S*-adenosyl-methionine. Phytochemistry 24: 1151–1155
7. HYODO H, K TANAKA, K WATANABE 1983 Wound-induced ethylene production and 1-aminocyclopropane-1-carboxylic acid synthase in mesocarp tissue of winter squash fruit. Plant Cell Physiol 24: 963–969
8. KENDE H, T BOLLER 1981 Would ethylene and 1-aminocyclopropane-1-carboxylate synthase in ripening tomato fruit. Planta 151: 476–481
9. KHANI-OSKOUEE S, JP JONES, RW WOODARD 1984 Stereochemical course of the biosynthesis of 1-aminocyclopropane-1-carboxylic acid. I. Role of the asymmetric sulfonium pole and the α-amino acid center. Biochem Biophys Res Commun 121: 181–187
10. KHANI-OSKOUEE S, K RAMALINGAM, D KALVIN, RW WOODARD 1987 Alternate substrates and inhibitors of 1-aminocyclopropane-1-carboxylic acid synthase. Bioorg Chem 15: 92–99
11. LAEMMLI UK 1970 Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680–685
12. LIEBERMAN M 1979 Biosynthesis and action of ethylene. Annu Rev Plant Physiol 30: 533–591
13. LIZADA MCC, SF YANG 1979 A simple and sensitive assay for 1-aminocyclopropane-1-carboxylic acid. Anal Biochem 100: 140–145
14. LUNDBLAD RL, CM NOYES 1984 *In* Chemical Reagent for Protein Modification, Vol I. CRC Press, Boca Raton, FL, pp 142–147
15. MIURA GA, PK CHIANG 1985 A radiosotope assay for 1-aminocyclopropane-

1-carboxylic acid synthase: *S*-adenosylhomocysteine analogs as inhibitor of the enzyme involved in plant senescence. Anal Biochem 147: 217–221
16. PRIVALLE LS, JS GRAHAM 1987 Radiolabeling of a wound-induced pyridoxal phosphate-utilizing enzyme: Evidence for its identification as ACC synthase. Arch Biochem Biophys 253: 333–340
17. RAMALINGAM K, K LEE, RW WOODARD, AB BLEECKER, H KENDE 1985 Stereochemical course of the reaction catalyzed by the pyridoxal phosphate-dependent enzyme 1-aminocyclopropane-1-carboxylate synthase. Proc Natl Acad Sci USA 82: 7820–7824
18. SATOH S, Y ESASHI 1986 Inactivation of 1-aminocyclopropane-1-carboxylic acid synthase of etiolated mung bean hypocotyl segments by its substrate, S-adenosyl-L-methionine. Plant Cell Physiol 27: 285–291
19. YOSHII H, H IMASEKI 1981 Biosynthesis of auxin-induced ethylene. Effects of indole-3-acetic acid, benzyladenine and abscisic acid on endogenous levels of 1-aminocyclopropane-1-carboxylic acid (ACC) and ACC synthase. Plant Cell Physiol 22: 369–379
20. YOSHII H, H IMASEKI 1982 Regulation of auxin-induced ethylene biosynthesis. Repression of inductive formation of 1-aminocyclopropane-1-carboxylate synthase by ethylene. Plant Cell Physiol 23: 639–649
21. YANG SF, NE HOFFMAN 1984 Ethylene biosynthesis and its regulation in higher plants. Annu Rev Plant Physiol 35: 155–189
22. YU Y, DO ADAMS, SF YANG 1979 1-Aminocyclopropanecarboxylate synthase, a key enzyme in ethylene biosynthesis. Arch Biochem Biophys 198: 280–286
23. YU Y, SF YANG 1980 Biosynthesis of wound ethylene. Plant Physiol 66: 281–285