Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 7930–7934, October 1990
Botany

Characterization and sequencing of the active site of 1-aminocyclopropane-1-carboxylate synthase

(*S*-adenosyl-L-methionine/pyridoxal phosphate/suicide inhibitor/*Malus sylvestris* Mill. and *Lycopersicon esculentum* Mill./fruit ripening)

WING-KIN YIP*, JIAN-GUO DONG*, JAMES W. KENNY†, GREGORY A. THOMPSON†, AND SHANG F. YANG*‡

*Department of Vegetable Crops, Mann Laboratory, University of California, Davis, CA 95616; and †Calgene Inc., 1920 Fifth Street, Davis, CA 95616

Contributed by Shang F. Yang, July 24, 1990

ABSTRACT **The pyridoxal phosphate (PLP)-dependent 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (*S*-adenosyl-L-methionine methylthioadenosine-lyase, EC 4.4.1.14), the key enzyme in ethylene biosynthesis, is inactivated by its substrate *S*-adenosylmethionine (AdoMet). Apple ACC synthase was purified with an immunoaffinity gel, and its active site was probed with NaB^3H_4 or Ado[^{14}C]Met. HPLC separation of the trypsin digest yielded a single radioactive peptide. Peptide sequencing of both ^{3}H- and ^{14}C-labeled peptides revealed a common dodecapeptide of Ser-Leu-Ser-Xaa-Asp-Leu-Gly-Leu-Pro-Gly-Phe-Arg, where Xaa was the modified, radioactive residue in each case. Acid hydrolysis of the ^{3}H-labeled enzyme released radioactive *N*-pyridoxyllysine, indicating that the active-site peptide contained lysine at position 4. Mass spectrometry of the ^{14}C-labeled peptide indicated a protonated molecular ion at *m/z* 1390.6, from which the mass of Xaa was calculated to be 229, a number that is equivalent to the mass of a lysine residue alkylated by the 2-aminobutyrate portion of AdoMet, as we previously proposed. These results indicate that the same active-site lysine binds the PLP and convalently links to the 2-aminobutyrate portion of AdoMet during inactivation. The active site of tomato ACC synthase was probed in the same manner with Ado [^{14}C]Met. Sequencing of the tomato active-site peptide revealed two highly conserved dodecapeptides; the minor peptide possessed a sequence identical to that of the apple enzyme, whereas the major peptide differed from the minor peptide in that methionine replaced leucine at position 6.**

The plant hormone ethylene regulates many aspects of plant growth and development. Adams and Yang (1) have established that ethylene is biosynthesized via the following sequence: methionine → AdoMet → 1-aminocyclopropane-1-carboxylic acid (ACC) → C_2H_4. Ethylene production in plant tissue is normally very low but is greatly promoted at certain developmental stages, such as fruit ripening, by auxin treatment or under certain environmental stresses (2). In all cases the increasing ethylene production results from increased synthesis of ACC from AdoMet catalyzed by ACC synthase (*S*-adenosyl-L-methionine methylthioadenosine-lyase, EC 4.4.1.14). Thus, ACC synthase is the rate-limiting enzyme in ethylene biosynthesis.

Based on the observations that the enzyme activity is inhibited by pyridoxal 5′-phosphate (PLP)-utilizing enzyme inhibitors and requires addition of exogenous PLP for maximal activity, ACC synthase is believed to be a PLP-utilizing enzyme (3). The proposed reaction mechanism involves the formation of a Schiff base between the PLP coenzyme and AdoMet, followed by an α,γ-elimination, yielding ACC and methylthioadenosine (1–4). Privalle and Graham (5) and Satoh and Yang (6) showed that when a partially purified ACC synthase preparation isolated from wounded tomato pericarp was incubated with NaB^3H_4 in the presence of PLP, a number of proteins including a 50-kDa protein were radiolabeled.

Another important characteristic of ACC synthase is that its substrate, AdoMet, serves as a suicide inactivator. Satoh and Yang (6) demonstrated that when a partially purified ACC synthase preparation isolated from tomato fruit was incubated with Ado[3,4-^{14}C]Met and the resulting protein was analyzed by SDS/PAGE, only one radioactive protein was observed. This protein was judged to be ACC synthase based on the observations that its molecular mass was 50 kDa and that it was specifically bound to a monoclonal antibody against ACC synthase prepared by Bleecker *et al.* (7). Later work showed that ACC synthase was also radiolabeled with Ado[*carboxyl*-^{14}C]Met but not with Ado[*methyl*-^{14}C]Met (8). Such an AdoMet-dependent radiolabeling has been used as a tool for confirming the identity of ACC synthase on SDS/PAGE (9).

Since ACC synthase is unstable and present in low concentration, even in those tissues where ethylene production is greatly induced, progress in the purification of this enzyme has been slow. Recently, ACC synthase has been purified from ripe and wounded tomato fruits (7, 9), wounded winter squash fruit (10), wounded zucchini fruit (11), auxin-treated mungbean hypocotyl (12), and ripe apple fruit (13). Without revealing any sequence information, Sato and Theologis (11) have reported isolation of a cDNA clone encoding the wound-induced zucchini ACC synthase.

In this study, we purified apple ACC synthase with an immunoaffinity gel, and the active site of the enzyme was radiolabeled with NaB^3H_4 or with Ado [*carboxyl*-^{14}C]Met. We report the isolation and sequence of the active-site peptide following trypsin digestion. Our results indicate that it is the same lysine residue at the active site that binds the PLP and covalently links to the 2-aminobutyrate portion of AdoMet during the inactivation and that the active site sequences§ of the apple and tomato enzymes are highly conserved.

EXPERIMENTAL PROCEDURES

Materials. ACC synthase was isolated from ripe apple (*Malus sylvestris* Mill.) fruits (13) or from ripe and wounded tomato (*Lycopersicon esculentum* Mill.) fruits (6) as described. Crude ACC synthase extracts from both sources were first purified with DEAE-Sepharose to about 100 units/mg of protein prior to the immunoaffinity purification. One unit of enzyme activity is defined as 1 nmol of ACC

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; PLP, pyridoxal 5′-phosphate; AdoMet, *S*-adenosyl-L-methionine.

‡To whom reprint requests should be addressed.

§The sequences reported in this paper have been deposited in the Protein Identification Resource (National Biomedical Research Foundation) data base (accession nos. A33103, B33103, and C33103).

produced per hr. Immunoaffinity agarose gel for apple ACC synthase was prepared by passing 2 ml of the ascitic fluid (monoclone line 6A10) containing anti-ACC synthase IgG to 2 ml of protein A-agarose (Pierce); the bound antibodies were then covalently linked to the protein A matrix with dimethyl pimelimidate (Pierce) by the method of Reeves *et al.* (14). Similarly, the immunoaffinity agarose for tomato ACC synthase was prepared by coupling anti-ACC synthase IgG isolated from an ascitic fluid (monoclone line 5b, a gift from Hans Kende, Michigan State University) to protein G-agarose (Pharmacia). N^{ϵ}-Pyridoxyllysine was synthesized from N^{α}-*tert*-butyloxycarbonyllysine (Sigma) and pyridoxal 5'-phosphate (Sigma) as described by Forrey *et al.* (15). After crystallization of N^{ϵ}-phosphopyridoxyl-N^{α}-*tert*-butyloxycarbonyllysine at −20°C, the compound was hydrolyzed in 6 M HCl at 110°C for 12 hr to yield N^{ϵ}-pyridoxyllysine, which was used as a standard. Radioactive Ado[*carboxyl*-^{14}C]Met (55 mCi/mmol; 1 Ci = 37 GBq) was obtained from New England Nuclear; NaB^3H_4 (14 Ci/mmol), from Research Products International; and *N*-tosyl-L-phenylalanyl chloromethyl ketone (TPCK)-treated trypsin, from Sigma.

Active-Site Labeling of ACC Synthase. For NaB^3H_4 reduction, a sample of ACC synthase preparation (50,000 units, containing 100 μg of ACC synthase protein) was passed through an immunoaffinity gel column (0.5-ml bed volume in 1-ml pipette tip). The affinity column was loaded and then washed with 10 mM sodium phosphate buffer (pH 7.0) containing 10 μM PLP. The immunoaffinity gel was then transferred to a 1.5-ml Eppendorf tube to which 25 mCi of NaB^3H_4 (14 Ci/mmol; 1 Ci = 37 GBq) was added. After 30 min at 0°C, 1 mg of unlabeled $NaBH_4$ was added, and incubation was continued for another 30 min. After the gel was washed with 20 mM NH_4HCO_3, the adsorbed radiolabeled ACC synthase was eluted with 4 bed volumes of 2% SDS in H_2O. For Ado[*carboxyl*-^{14}C]Met labeling, a sample of ACC synthase (30,000 units containing 60 μg of ACC synthase) was similarly passed through a column containing 0.3 ml of immunogel. After the gel was washed extensively with 100 mM Hepes buffer (pH 8.5) containing 10 μM PLP, 10 μCi of Ado[^{14}C]Met (55 mCi/mmol), which had been preheated at 100°C for 7 min in 0.05 M H_2SO_4 (6), was added to the immunogel. After 6 hr with gentle shaking at 30°C, 600 nmol of preheated AdoMet was added, and the incubation was allowed to proceed for another 6 hr.

Trypsin Digestion and Peptide Separation. Radiolabeled ACC synthase was eluted from the immunoaffinity column with 2% SDS, lyophilized, and suspended in acetone/acetic acid/triethylamine/H_2O, 86:5:5:4 (vol/vol). In this buffer system, SDS forms an ion pair with triethylamine and dissolves in acetone. This causes the protein to precipitate (16). After two extractions the protein was washed with acetone and dried under vacuum. The dried ACC synthase (*ca.* 60–100 μg) was suspended in 2 M urea containing 50 mM NH_4HCO_3 (pH 8.0), and TPCK-treated trypsin was added to yield a final 5% trypsin-to-protein ratio. The total volume of the digestion mixture was kept under 100 μl, and the mixture was incubated at 37°C for 12 hr. The tryptic peptides were separated on a C_4 reverse-phase HPLC column with a linear acetonitrile gradient with a flow rate of 1 ml/min: the first dimension was 10–40% acetonitrile in 20 mM ammonium acetate (pH 6.0) for 80 min; the second dimension was 10–40% acetonitrile in 0.1% CF_3COOH(pH < 2.0) for 50 min; and the third dimension was 10–40% acetonitrile in 0.1% CF_3COOH (pH < 2.0) for 80 min. HPLC was performed with a Beckman HPLC system model 110A on a Bio-Rad Hi-Pore reverse-phase column (RP-304, 250 mm × 4.6 mm) and a Beckman model 160 absorbance detector fitted with a 214-nm filter. Fractions from the column were manually collected, and the radioactivity was determined by liquid scintillation counting.

Peptide Sequencing. Edman degradation of the purified radiolabeled peptide was performed by using an Applied Biosystems model 477A protein sequencer, with on-line separation of phenylthiohydantoin-amino acids on an Applied Biosystems model 120 phenylthiohydantoin-amino acid analyzer. To detect the radioactivity release, fractions (30%) from each sequencer cycle were determined in a liquid scintillation counter. The molecular mass of the purified peptide was determined by liquid secondary-ion mass spectrometry by using a Kratos MS50S double-focusing mass spectrometer equipped with a Cs ion gun and a high-field magnet (17) at the Mass Spectrometry Facility of the University of California, San Francisco.

Identification of Labeled N^{ϵ}-Pyridoxyllysine. The radiolabeled ACC synthase resulting from NaB^3H_4 reduction was hydrolyzed with 6 M HCl at 110°C under N_2 for 12 hr. The hydrolysate was dried *in vacuo*, and the residue was dissolved in 2 ml of H_2O. The amino acid fraction was adsorbed onto a small column of Dowex 50 cation-exchange resin (H^+ form) and eluted with 2 M NH_4OH. After the NH_4OH was evaporated, the residue was dissolved in a minimal amount of H_2O. A sample of radioactive ACC synthase (30,000 dpm) was hydrolyzed with 6 M HCl at 110°C for 12 hr, and its hydrolysis products were then analyzed with authentic N^{ϵ}-pyridoxyllysine by descending paper cochromatography developed in 1-butanol/pyridine/acetic acid/H_2O, 30:20:6:24 (vol/vol), and paper coelectrophoresis at pH 6.5 with a solvent system of pyridine/acetic acid/H_2O, 10:0.4:90 (vol/vol) (15). Standard N^{ϵ}-pyridoxallysine on paper chromatogram and electrophoretogram was located by fluorescence under UV lamp; radioactive zones were determined by liquid scintillation counting of the filter paper cut from different positions.

Gel Electrophoresis and Fluorography. SDS/PAGE was conducted with 10% gel by the methods of Laemmli (18). Gels were stained with Coomassie blue and prepared for fluorography by soaking in Fluoro-Hance (Research Products International). After drying, they were exposed to Kodak XAR-5 x-ray film at −80°C.

RESULTS AND DISCUSSION

Because of the low abundance and apparent lability of ACC synthase (7, 9), isolation of the native enzyme in sufficient amounts for structural analysis by classical protein purification techniques has proved to be a difficult task. An alternative approach is the radiolabeling of the enzyme coupled with immunoaffinity purification. Since ACC synthase is a PLP enzyme (1–5, 9) and is inactivated by its substrate AdoMet, ACC synthase has been shown to be radiolabeled by NaB^3H_4 reduction or by Ado[^{14}C]Met during the suicide inactivation. While the radiolabeling of ACC synthase with AdoMet appears to be specific (6, 9, 13), radiolabeling with NaB^3H_4 is not (5, 6), presumably because many other PLP-dependent enzymes are present in the crude or partially purified preparations. In this study, immunoaffinity agarose gels prepared from anti-ACC synthase monoclonal antibodies (7, 13) were used to specifically adsorb ACC synthase from a partially purified apple or from tomato enzyme preparations (purity *ca.* 100 units/mg of protein). After the unbound proteins were washed off, ACC synthase was radiolabeled while it was still bound to the immunoaffinity gel. This labeling technique is possible presumably because the monoclonal antibodies used in these experiments bind to an epitope of the enzyme that is different from the substrate and PLP binding sites (7, 13). Fig. 1 shows the SDS/PAGE profile of an apple enzyme preparation after NaB^3H_4 reduction and immunoaffinity gel purification. Note that there was only one radiolabeled band that coincided with the major protein band eluted from the immunoaffinity gel. This protein

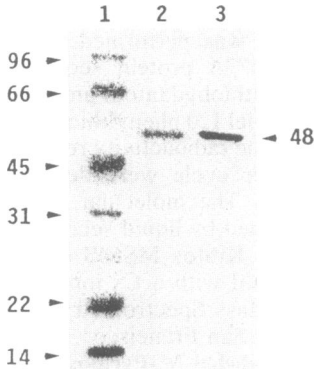

FIG. 1. SDS/PAGE analysis of apple ACC synthase radiolabeled with NaB^3H_4. A sample of partially purified ACC synthase (3000 units) was passed through an immunoaffinity gel, and the enzyme, while bound to the gel, was radiolabeled with NaB^3H_4. After extensive washing, proteins were eluted from the gel with 2% SDS. Lanes: 1, molecular mass standards; 2, Coomassie blue staining; 3, fluorogram of lane 2 for 6 hr. Molecular mass is shown in kDa.

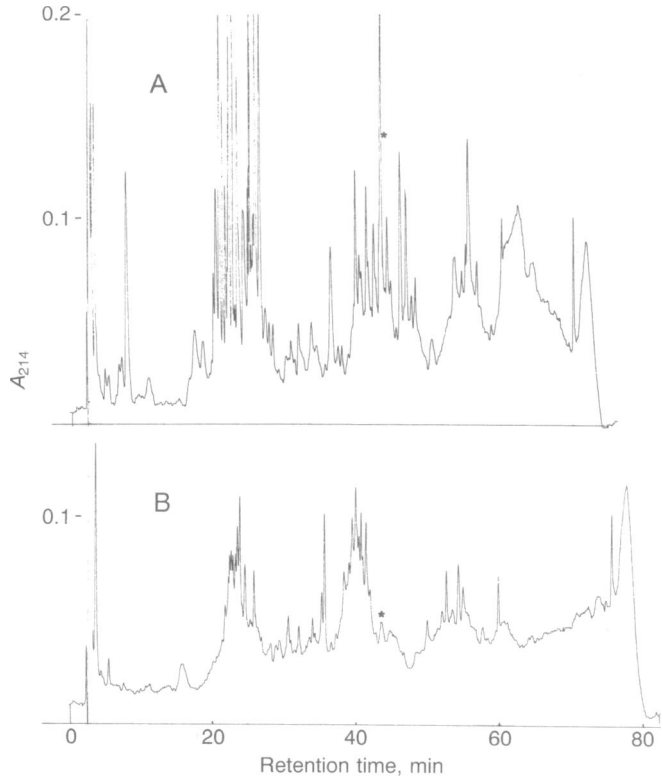

FIG. 2. HPLC separation of trypsin digests of labeled apple ACC synthase. (*A*) Partially purified ACC synthase (50,000 units) was purified on an immunoaffinity column, and the enzyme, while bound to the affinity gel, was radiolabeled by reduction with NaB^3H_4. (*B*) Partially purified ACC synthase (30,000 units) was purified on an immunoaffinity column, and the enzyme bound to the gel was radiolabeled with Ado[*carboxyl*-^{14}C]Met. After elution with 2% SDS, the proteins were digested with trypsin, and the resulting peptides were separated by HPLC. The radioactive fractions are indicated by asterisks. For *A*, the tritiated peptide was further purified by second- and third-dimensional HPLC, and for *B*, ^{14}C-labeled peptide was further purified by a second-dimensional HPLC step as described in the text.

was judged to be ACC synthase based on its molecular mass of 48 kDa and on the observations that it was specifically radiolabeled with AdoMet and specifically bound to a monoclonal antibody against ACC synthase. After trypsin digestion of this radiolabeled protein, only a single radiolabeled peptide was isolated from HPLC (Fig. 2). Edman degradation of this labeled peptide revealed a 12-amino acid sequence, Ser-Leu-Ser-Xaa$_i$-Asp-Leu-Gly-Leu-Pro-Gly-Phe-Arg, where Xaa$_i$ in cycle 4 was radioactive and did not match to any standards. It is logical to assume that Xaa$_i$ is N^{ϵ}-phosphopyridoxyllysine. To ascertain this, the tritiated ACC synthase was hydrolyzed in 6 M HCl at 110°C for 12 hr, and the resulting radioactive product was analyzed by paper coelectrophoresis and cochromatography. Indeed, it yielded only a single radioactive compound, which comigrated with the authentic N^{ϵ}-pyridoxyllysine in both paper chromatography and paper electrophoresis (data not shown). These data indicate that residue 4 of the tryptic peptide is a radioactively modified lysine (Table 1). A common sequence of Ser-Xaa-Xaa-Lys (or Thr-Xaa-Xaa-Lys in some cases) has been found in a number of PLP-utilizing enzymes, where the hydroxyl group of the serine (or threonine) residue is thought to bind the 5′-phosphate of the PLP coenzyme through the hydrogen bonding interaction (19–21). The sequence of Ser-Leu-Ser-Lys found in apple ACC synthase is also consistent with such a pattern. Comparisons of the ACC synthase active site with that of other PLP-utilizing enzymes indicate that cytosolic and mitochondrial aspartate aminotransferases (EC 2.6.1.1) from various species (22–27) and histidinol-phosphate aminotransferases (EC 2.6.1.9) from yeast and *Salmonella typhimurium* (20, 28) show 50–60% sequence homology with the apple ACC synthase active-site peptide.

It has been shown that the 2-aminobutyrate portion of AdoMet became specifically and covalently linked to ACC synthase during the inactivation reaction (6, 8, 9, 13, 29). To elucidate to which amino acid residue the 2-aminobutyrate moiety of AdoMet links, we had similarly radiolabeled ACC synthase with radioactive AdoMet. When a partially purified apple ACC synthase preparation was incubated with Ado[*carboxyl*-^{14}C]Met, only one radioactive protein band of 48 kDa was detected on SDS/PAGE (13). This radioactive protein was specifically bound to the immunoaffinity gel and was eluted with 2% SDS (ref. 13; data not shown). Following trypsin digestion, the resulting peptides were separated by HPLC as shown in Fig. 2. There was only one radiolabeled peptide isolated with a retention time similar to that of the tritiated peptide derived from NaB^3H_4 labeling. Edman degradation of this labeled peptide revealed the same dodecapeptide Ser-Leu-Ser-Xaa$_{ii}$-Asp-Leu-Gly-Leu-Pro-Gly-Phe-Arg, where the unidentified radioactive phenylthiohydantoin-amino acid derivative (Xaa$_{ii}$) was also released in cycle 4. These results indicate that residue 4 of this dodecapeptide derived from AdoMet labeling could be a lysine derivative, as was found in the dodecapeptide derived from NaB^3H_4 reduction.

When this purified dodecapeptide was subjected to liquid secondary-ion mass spectrometry, a protonated molecular

Table 1. Sequence homology of the active-site peptide of apple and tomato ACC synthase

Source	Sequence
Apple	H_2N-Ser-Leu-Ser-Lys*-Asp-Leu-Gly-Leu-Pro-Gly-Phe-Arg-COOH
Tomato	H_2N-Ser-Leu-Ser-Lys*-Asp-Met-Gly-Leu-Pro-Gly-Phe-Arg-COOH
	H_2N-Ser-Leu-Ser-Lys*-Asp-Leu-Gly-Leu-Pro-Gly-Phe-Arg-COOH

*Location of bound radioactivity when the enzyme was radiolabeled with NaB^3H_4 or Ado[*carboxyl*-^{14}C]Met.

ion at m/z 1390.6 was obtained. Subtracting the mass of the other known amino acid residues, the mass of Xaa_{ii} was calculated to be 229, which is equivalent to the mass of lysine or glutamine alkylated by a vinylglycine group. Since an alkylation of glutamine by vinylglycine is chemically unlikely, a lysine residue at position 4 is assumed. In addition, purified ACC synthase without the AdoMet treatment yielded the octapeptide Asp-Leu-Gly-Leu-Pro-Gly-Phe-Arg after trypsin digestion. Since this peptide contained the last eight residues of the labeled peptide, it is probable that the amino acid residue preceding aspartic acid is a lysine, which forms a susceptible site for trypsin cleavage. The above results strongly indicate that residue 4 of the labeled tryptic peptide derived from suicide inactivation by AdoMet is lysine.

The above results indicate that it is the same lysine (position 4 of the tryptic peptide) at the active site that binds the PLP in native enzyme and covalently links to the 2-aminobutyrate portion of AdoMet during the inactivation process. Fig. 3 summarizes a postulated scheme for both labeling mechanisms. In native form the ε-NH_2 of the active site lysine forms an aldimine bond with the aldehyde group of PLP, and this aldimine linkage is readily reduced to the hydrolytically stable secondary amine by $NaBH_4$. When the substrate AdoMet is available, the "trans-schiffization" (30) occurs, and the catalysis proceeds via an α,γ-elimination of AdoMet to form ACC. However, in 1 of 30,000 catalytic cycle, a β,γ-elimination occurs, in which a vinylglycine intermediate is formed (8, 29); a nucleophilic attack by the ε-NH_2 of the active site lysine on the electrophilic β-carbon of the methyldehydroalanine–PLP complex yields a product in which the ε-NH_2 of the active site lysine is alkylated. However, a definitive proof of the product to be N^{ε}-(1-methyl-2-amino-2-carboxyethyl)lysine as shown in Fig. 3 awaits the separation of this peptide in sufficient quantity for further chemical identification.

Although ACC synthases isolated from various species exhibits similar K_m values and cofactor requirements and are inactivated by their substrate AdoMet, they appear to differ in their immunological and physicochemical properties. For example, the monoclonal antibody raised against ripening- and wound-induced tomato ACC synthase (7) failed to cross-react with apple fruit enzyme (31), with the wound-induced enzyme from winter squash, and with auxin-induced enzyme

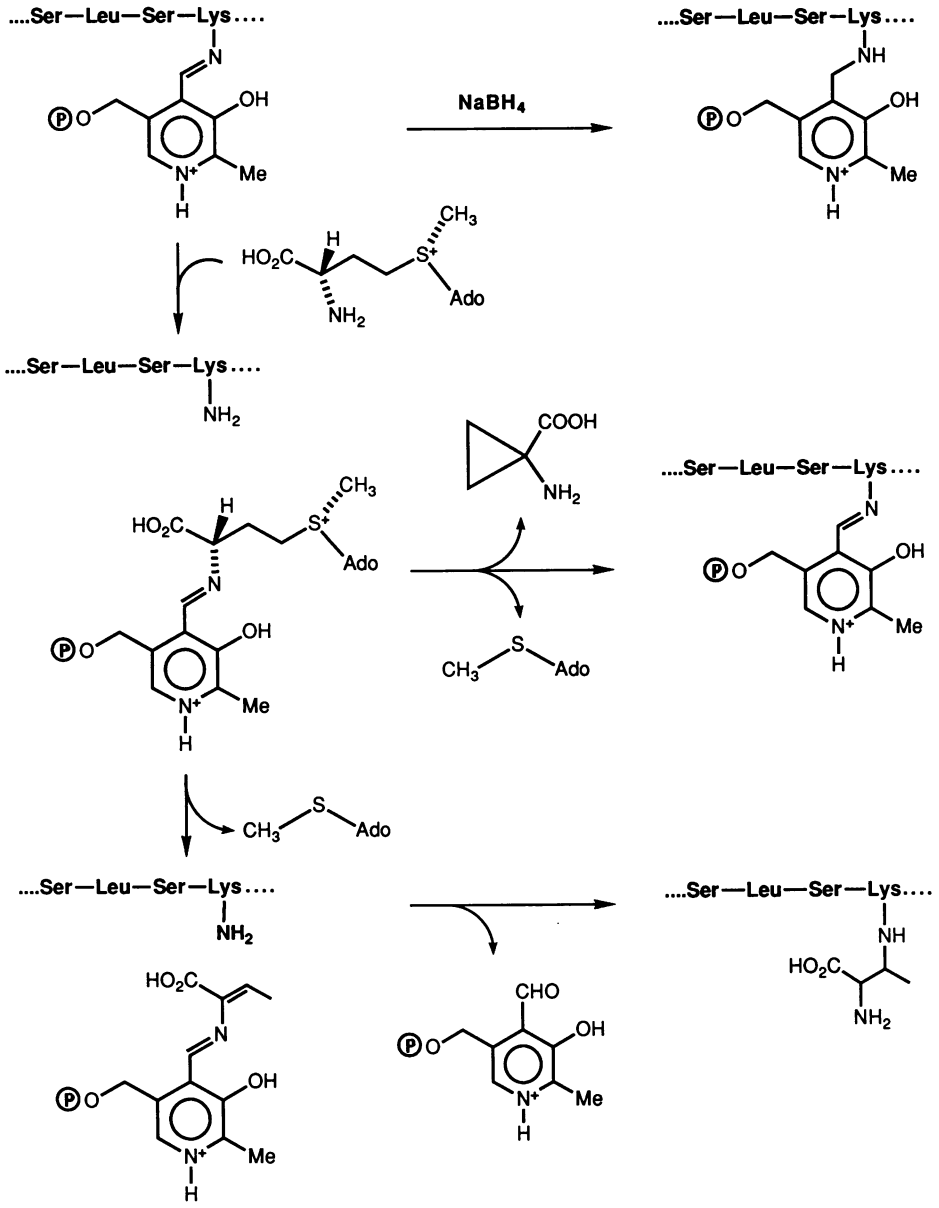

FIG. 3. Reaction mechanisms of ACC synthase radiolabeled by NaB^3H_4 and by Ado[^{14}C]Met.

from pea seedlings (H. Kende, private communication); also the polyclonal antibodies against wound-induced ACC synthase from winter squash did not recognize the enzyme from auxin-induced mungbean seedlings (32) or from ripe apple fruits (unpublished results). Our monoclonal antibodies against ripening-induced apple enzyme failed to recognize the ripening- and wound-induced tomato fruit enzyme (unpublished results). These observations lead to the suggestion that there are multiple forms of ACC synthase (32). In this study, we have also isolated the active site peptide of tomato ACC synthase labeled with AdoMet. Peptide sequencing of the tomato active-site peptide revealed two highly conserved dodecapeptides; these sequences are identical except that both methionine and leucine appeared in position 6. The amount of phenylthiohydantoin-methionine was about 3 times that of phenylthiohydantoin-leucine, indicating that the methionine-containing peptide is the major one. As in apple enzyme, the radioactive residue was released in cycle 4. The results are summarized in Table 1.

Whereas apple enzyme was isolated from ripe fruits, tomato enzyme was isolated from ripe, wounded fruits. This observation prompted us to hypothesize that there are two ACC synthase genes in tomato: one is specifically activated by ripening and the other by wounding. It is probable that the immunological and physicochemical properties of these two isozymes are so similar that these two enzymes are copurified during the purification procedures. If this predication is correct, we may further speculate that the ripening-activated tomato ACC synthase contains leucine at position 6 of its active-site tryptic peptide, whereas the wound-activated enzyme contains methionine.

After this work was completed, H. Imaseki (personal communication) of Nagoya University and D. Van Der Straeten (33) informed us that they had recently isolated and sequenced ACC synthase cDNA clones from wounded winter squash fruit and wounded tomato fruit, respectively. Indeed, our active-site peptide sequence of Ser-Leu-Ser-Lys-Asp-Met-Gly-Leu-Pro-Gly-Phe-Arg can be deduced from their cDNA sequences. Thus, their cDNA sequence fully confirmed our active-site peptide sequence, including the identity of lysine at position 4, and vice versa. In both cDNA clones, the predicted amino acid residue corresponding to position 6 of tryptic active-site peptide is methionine. This is in agreement with our hypothesis, because both cDNA clones were isolated from wounded fruit tissues.

Although apple and tomato enzymes differed in immunoproperties, their active-site sequence is either identical or highly conserved.

We are grateful to Dr. Hans Kende (Michigan State University) for generously providing us ascitic fluid containing anti-tomato ACC synthase monoclonal antibodies and to Dr. Hans Kende and Dr. Christopher T. Walsh (Harvard Medical School) for reviewing this manuscript. We acknowledge Dr. K. F. Medzihradszky of the Mass Spectrometry Facility (supported by National Institutes of Health Grant DRR RR01614 to Dr. A. L. Burlingame), University of California, San Francisco, for the mass spectrometry analysis. This work was supported by grants to S.F.Y. from the National Science Foundation (INT-8915155 and DCB-9004129).

1. Adams, D. O. & Yang, S. F. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 170–174.
2. Yang, S. F. & Hoffman, N. E. (1984) *Annu. Rev. Plant Physiol.* **35,** 155–189.
3. Yu, Y.-B., Adams, D. O. & Yang, S. F. (1979) *Arch. Biochem. Biophys.* **198,** 280–286.
4. Ramalingam, K., Lee, K.-M., Woodard, R. W., Bleecker, A. B. & Kende, H. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 7820–7824.
5. Privalle, L. S. & Graham, J. S. (1987) *Arch. Biochem. Biophys.* **253,** 333–340.
6. Satoh, S. & Yang, S. F. (1988) *Plant Physiol.* **88,** 109–114.
7. Bleecker, A. B., Kenyon, W. H., Somerville, S. C. & Kende, H. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 7755–7759.
8. Sato, S. & Yang, S. F. (1989) *Arch. Biochem. Biophys.* **271,** 107–112.
9. Van Der Streaten, D., Van Wiermeersch, L., Goodman, H. M. & Van Montagu, M. (1989) *Eur. J. Biochem.* **182,** 639–647.
10. Nakajima, N., Nakagawa, N. & Imaseki, H. (1988) *Plant Cell Physiol.* **29,** 989–998.
11. Sato, T. & Theologis, A. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 6621–6625.
12. Tsai, D.-S., Arteca, R. N., Bachman, J. M. & Phillips, A. T. (1988) *Arch. Biochem. Biophys.* **264,** 632–640.
13. Yip, W.-K., Dong, J.-G. & Yang, S. F. (1990) *Plant Physiol.*, in press.
14. Reeves, H. C., Heeren, R. & Molloy, P. (1981) *Anal. Biochem.* **115,** 194–196.
15. Forrey, A. W., Olsgaard, R. B., Nolan, C. & Fischer, E. H. (1971) *Biochimie* **53,** 269–281.
16. Henderson, S., Oroszlan, S. & Konigberg, W. (1979) *Anal. Biochem.* **93,** 153–157.
17. Falick, A. M., Wang, G. H. & Walls, F. C. (1986) *Anal. Chem.* **58,** 1308–1311.
18. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
19. Ford, G. C., Eichele, G. & Jansonious, J. N. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 2559–2563.
20. Hsu, L. C., Okamato, M. & Snell, E. E. (1989) *Biochimie* **71,** 4777–4790.
21. Tanase, S., Kojima, H. & Morino, Y. (1979) *Biochemistry* **18,** 3002–3007.
22. Kagamiyama, H., Sakakibara, R., Wada, H., Tanase, S. & Morino, Y. (1977) *J. Biochem.* **82,** 291–294.
23. Kondo, K., Wakabayashi, S., Yagi, T. & Kagamiyama, H. (1984) *Biochem. Biophys. Res. Commun.* **122,** 62–67.
24. Mattingly, J. R., Jr., Rodriguez-Berrocal, F. J., Gordon, J., Iriarte, A. & Martinez-Carrion, M. (1987) *Biochem. Biophys. Res. Commun.* **149,** 859–865.
25. Ovchinnikov, Yu., Egorov, C. A., Aldanova, N. A., Feigina, M. Yu., Lipkin, V. M., Abdulaev, N. G., Grishin, E. V., Kiselev, A. P., Modyanov, N. N., Braunstein, A. E., Polyanovskky, O. L. & Nosikov, V. V. (1973) *FEBS Lett.* **29,** 31–34.
26. Shlyapnikov, S. V., Myasnikov, A. N., Severin, E. S., Myagkova, M. A., Torchinsky, Y. M. & Braunstein, A. E. (1979) *FEBS Lett.* **106,** 385–388.
27. Teranishi, H., Kagamiyama, H., Teranishi, K., Wada, H., Yamano, T. & Morino, Y. (1978) *J. Biol. Chem.* **253,** 8842–8847.
28. Nishiwaki, K., Hayashi, N., Irie, S., Chung, D-H., Harashima, S. & Oshima, Y. (1987) *Mol. Gen. Genet.* **208,** 159–167.
29. Satoh, S. & Yang, S. F. (1989) *Plant Physiol.* **91,** 1036–1039.
30. Cordes, E. H. & Jenck, W. P. (1962) *Biochemistry* **1,** 773–778.
31. Dong, J.-G., Yip, W.-K. & Yang, S. F. (1988) *Plant Physiol.* **86,** S-375 (abstr.).
32. Nakakawa, N., Nakajima, N. & Imaseki, H. (1988) *Plant Cell Physiol.* **29,** 1255–1259.
33. Van Der Streaten, D., Van Wiemeersch, L., Goodman, H. M. & Van Montagu, M. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 4859–4863.