Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 7820–7824, December 1985
Biochemistry

Stereochemical course of the reaction catalyzed by the pyridoxal phosphate-dependent enzyme 1-aminocyclopropane-1-carboxylate synthase

(*S*-adenosylmethionine/deuterium-labeling/vinylglycine/enzyme mechanism)

KONDAREDDIAR RAMALINGAM†, KANG-MAN LEE†, RONALD W. WOODARD†‡, ANTHONY B. BLEECKER§, AND HANS KENDE§

†College of Pharmacy, The University of Michigan, Ann Arbor, MI 48109; and the §Michigan State University–Department of Energy Plant Research Laboratory, Michigan State University, East Lansing, MI 48824

Communicated by Anton Lang, July 15, 1985

ABSTRACT **(±)-*S*-adenosyl-DL-(3*R**,4*S**)-[3,4-$^2H_2$]-methionine [a 1:1 mixture of (3*R*,4*S*) and (3*S*,4*R*)] and (±)-*S*-adenosyl-DL-(3*R**,4*R**)-[3,4-$^2H_2$]methionine [a 1:1 mixture of (3*R*,4*R*) and (3*S*,4*S*)] were synthesized from (*Z*)- and (*E*)-[1,2-$^2H_2$]ethene, respectively. Key steps in the synthesis were the antiperiplanar addition of methanesulfenyl chloride to (*Z*)-[1,2-$^2H_2$]ethene, to give a 1:1 mixture of (*R*,*R*)- and (*S*,*S*)-1-chloro-2-(methylthio)[1,2-$^2H_2$]ethane, followed by alkylation with sodium acetamidomalonate and hydrolysis to give an equal mixture of four stereoisomers of [3,4-$^2H_2$]methionine [(2*R*, 3*R*,4*S*), (2*R*,3*S*,4*R*), (2*S*,3*R*,4*S*), and (2*S*,3*S*,4*R*)]. The other four stereoisomers of [3,4-$^2H_2$]methionine were prepared from (*E*)-(1,2-$^2H_2$]ethene. The two sets of stereoisomers of [3,4-$^2H_2$]methionine were chemically converted to *S*-adenosylhomocysteine, methylated to give the corresponding (±)-*S*-adenosyl-DL-methionines, and then incubated with 1-aminocyclopropane-1-carboxylate synthase partially purified from tomato (*Lycopersicon esculentum*, L.) pericarp tissue. The stereochemistry of the resulting samples of 1-aminocyclopropane-1-carboxylic acid was determined by comparison with the ^1H NMR of the chemically synthesized and regio- and stereospecifically deuterated compound. The results indicate that the hydrogens at the β carbon of the methionine portion of *S*-adenosylmethionine do not participate in the reaction and that the ring closure occurs with inversion of configuration at the γ carbon of the methionine portion of *S*-adenosylmethionine, probably through a direct S_N2-type displacement of the 5′-methylthio-5′-deoxyadenosine moiety by a carbanion equivalent formed at the α carbon of the methionine portion of *S*-adenosylmethionine.**

Although the compound was first synthesized by Perkin in 1884 (1), it was not until 1957 that Burroughs (2) isolated the cyclopropanoid amino acid 1-aminocyclopropane-1-carboxylic acid (ACC) from cider apples and perry pears. In 1979, Adams and Yang (3) demonstrated that this cyclic amino acid was the key intermediate in the bioconversion of methionine to ethylene, an endogenous plant hormone responsible for fruit-ripening as well as a number of other regulative plant processes (4). It was suggested that conversion of *S*-adenosyl-L-methionine (AdoMet), a biologically activated form of methionine (5), to ACC was the rate-limiting reaction in the biosynthesis of ethylene (6). The enzyme that catalyzes the cyclization, ACC synthase, was first isolated from tomato pericarp by Boller *et al.* (7) and was shown to require pyridoxal phosphate as cofactor (8).

Enzymes that contain pyridoxal-*P* as the prosthetic group catalyze a variety of reactions at the α, β, and γ carbons of α-amino acids by one of several basic mechanisms (9). With the exception of reactions occurring at the γ carbon, the mechanism and stereochemistry of these enzyme reactions have been studied extensively (10). Within the set of pyridoxal-*P* enzymes catalyzing reactions at the γ carbon, ACC synthase seems to represent a unique subset. In the γ-elimination reactions studied to date, the accepted mechanism is as follows: the loss of a γ-carbon substituent is generally facilitated by tautomerization of the aldimine to the ketimine and subsequent formation of a β-carbanion that can assist in the elimination of the γ-leaving group, thus forming a β,γ-unsaturated imine intermediate (11). The presence of this vinylglycine-type intermediate is consistent with experimental evidence obtained from previously studied γ-elimination reactions (12–14) in that one of the β-hydrogens is stereospecifically exchanged. However, the conversion of this β,γ-unsaturated imine to ACC by ACC synthase (see Fig. 4, mechanism A), if concerted, would violate Baldwin's rules, which disallow the concerted process of three-member-ring formation by addition of a carbanion to a double bond in an endo fashion (15).

To gain insight into the mechanism of the reaction catalyzed by ACC synthase, we have initiated studies to probe the stereochemical events at the α, β, and γ carbons of the methionine portion of AdoMet during the cyclization process. The main stereochemical questions are (*a*) does replacement of the methine hydrogen of the α carbon by the new carbon bond proceed in an inversion or retention mode, (*b*) does the β carbon participate in the reaction mechanism and, if so, is the *pro-R* or *pro-S* hydrogen removed and does reprotonation of the β,γ-unsaturated imine occur on the *re* or *si* face of the *cisoid* or *transoid* form, and (*c*) does the displacement of the 5′-methylthio-5′-deoxyadenosine moiety at the γ carbon proceed with inversion or retention? In this paper, we report results that answer questions *b* and *c*.

MATERIALS AND METHODS

General. Organic chemicals were purchased from Aldrich; biochemicals, from Sigma; and *E*- and *Z*-[1,2-$^2H_2$]ethene (98.9% ^2H and 96.5% ^2H, respectively), from Merck Sharp & Dohme. All materials were used without further purification.

Melting points were determined with a Thomas–Hoover capillary melting-point apparatus and are uncorrected. TLC separations were carried out on Analtech silica gel plates. Evaporation under reduced pressure refers to solvent removal on a Buchi rotary evaporator at or below 40°C. ^1H-NMR spectra were recorded on an IBM WP-270 (270 MHz) spectrometer. Chemical shifts are reported in ppm from

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.

Abbreviation: ACC, 1-aminocyclopropane-1-carboxylic acid.

‡To whom reprint requests should be addressed.

internal 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt in ^2H_2O.

The DL-(3*R**,4*R**)- and DL-(3*S**,4*R**)-[3,4-$^2H_2$]methionine samples were synthesized by the method of Billington and Golding (16) from *E*- and *Z*-[1,2-$^2H_2$]ethene, respectively. 1-Amino-(2*S**,3*S**)-[2,3-$^2H_2$]cyclopropane-1-carboxylic acid (*trans*-ACC) and 1-amino-(2*S**,3*R**)-[2,3-$^2H_2$]cyclopropane-1-carboxylic acid (*cis*-ACC) were synthesized by the method of Ramalingam *et al.* (17) from *Z*- and *E*-[1,2-$^2H_2$]ethene, respectively.

S-Adenosyl-DL-(3R*,4R*)-[3,4-$^2H_2$]Homocysteine. Small pieces of sodium were added to a stirred solution of DL-(3*R**,4*R**)-[3,4-$^2H_2$]methionine (16) (75 mg, 0.5 mmol) in liquid NH_3 (25 ml) until the blue color persisted for 20 min. To this solution was added 5'-chloro-5'-deoxyadenosine (18) (189 mg, 0.66 mol) in portions, and the resulting mixture was stirred at −40°C for 6 hr. The NH_3 was allowed to evaporate over 20–30 min with the last traces being removed under reduced pressure. The residue was dissolved in water (4 ml) and the aqueous solution was heated to reflux for 4 hr. The reaction mixture was adjusted to pH 6.5 with 1 M HCl and applied to a 90-ml column of Dowex 50X4-200 (NH_4^+ form). The column was washed with water (150 ml), and then adsorbed material was eluted with 1 M NH_4OH; 2-ml fractions were collected. Fractions 22–30, containing the product, were pooled and lyophilized. The crude product was purified by column chromatography on silica gel (9.0 g, Kieselgel 60, 70–230 mesh), using elution with butanol/acetic acid/water (13:2:5, vol/vol) to remove unreacted 5'-chloro-5'-deoxyadenosine. Two-milliliter fractions were collected, those showing a positive reaction with ninhydrin (fractions 30–56) were combined, and the solvent was removed under reduced pressure to yield a colorless solid. The product was homogeneous on TLC [silica gel, butanol/acetic acid/water (13:2:5)]. The product was further purified by ion-exchange chromatography on a 15-ml column of Dowex 50X4-200 (NH_4^+): the column was washed with water to remove traces of acetic acid, and the purified product was eluted with 1 M NH_4OH. Fractions containing the product were pooled and lyophilized to give a fluffy white solid (yield 55%): mp 216–218°C; ^{1}H-NMR (^2H_2O) δ 8.37 (s, 1, C_8 H), 8.2 (s, 1, C_2 H), 6.1 (d, 1, J = 5.0 Hz, C_1 H), 4.88 (t, 1, J = 5.5 Hz, C_2 H), 4.48 (t, 1, J = 5.0 Hz, C_3 H), 4.41 (m, 1, C_4 H), 3.89 (d, 1, J = 6.3 Hz, C_α H), 3.13 and 3.04 (m, 2, C_5 H), 2.75 (m, 1, C_γ H), 2.21 and 2.14 (t, 1, J = 7.7 Hz, C_β H).

S-Adenosyl-DL-(3S*,4R*)-[3,4-$^2H_2$]Homocysteine. This compound was prepared from DL-(3*S**,4*R**)-[3,4-$^2H_2$]methionine (16) in a manner identical with that used to prepare the compound described above. Yield was 42%; mp 216–218°C, ^{1}H-NMR (^2H_2O) δ 8.35 (s, 1, C_8 H), 8.19 (s, 1, C_2 H), 6.1 (d, 1, J = 5.0 Hz, C_1 H), 4.87 (t, 1, J = 5.2 Hz, C_2 H), 4.48 (t, 1, J = 5.0 Hz, C_3 H), 4.40 (m, 1, C_4 H), 3.9 (d, 1, J = 6.0 Hz, C_α H), 3.12 and 3.05 (m, 2, C_5 H), 2.75 (m, 1, C_γ H), 2.21 and 2.14 (t, 1, J = 7.5 Hz, C_β H).

(±)-S-Adenosyl-DL-(3R*,4R*)-[3,4-$^2H_2$]Methionine (*trans*-AdoMet). Silver acetate (8.3 mg, 0.05 mmol) and iodomethane (0.2 ml, 25 mmol) were added to a solution of *S*-adenosyl-DL-(3*R**,4*R**)-[3,4-$^2H_2$]homocysteine (10.0 mg, 0.025 mmol) in 0.5 ml of acetic acid/formic acid (1:1, vol/vol), and the mixture was stirred at room temperature for 12 hr in the dark. The resulting slurry was centrifuged to remove silver iodide, and the acetic acid/formic acid solution was diluted with dry ether (20 ml). The ether solution was decanted from the oily precipitate and the precipitate was dissolved in water, filtered, and freeze-dried to give 7.8 mg (75% yield) of the product.

(±)-S-Adenosyl-DL-(3S*,4R*)-[3,4-$^2H_2$]Methionine (*cis*-AdoMet). Conversion of *S*-adenosyl-DL-(3*S**,4*R**)-[3,4-$^2H_2$]homocysteine into *cis*-AdoMet was accomplished (78% yield) by the same methods employed in the synthesis of the 3*R**,4*R** analogue.

Enzyme Purification. ACC synthase was extracted from tomato pericarp tissue according to Boller *et al.* (7), with the following modifications. Pericarp (1 kg) was incubated as described (19) overnight with 100 mM LiCl to induce ACC synthase formation and then homogenized with a Polytron, using an extraction buffer containing 100 mM potassium phosphate buffer (pH 8), 1 mM DL-dithiothreitol, 5 μM pyridoxal-*P*, and 5% (wt/vol) polyvinylpyrrolidone. Partial purification of the enzyme was achieved according to Acaster and Kende (20). A 40%–95% ammonium sulfate cut of the cleared homogenate was loaded onto a phenyl-Sepharose column (1.5 × 30 cm) in 12% ammonium sulfate. After elution from the column with 0.1 M NaCl, the enzyme was concentrated and stored at −80°C until used. A yield of 3000–6000 units (1 unit = 1 nmol of product formed per hour) of activity was routinely obtained, with a specific activity of 400–500 units/mg of protein.

Enzyme Incubation. *trans*-AdoMet (4 μmol) and *cis*-AdoMet (5 μmol) were incubated at 30°C for 3–4 hr with 6000 and 3000 units of activity, respectively. Initial reaction mixtures contained 150 μM AdoMet, 5 μM pyridoxal-*P*, 1 μM dithiothreitol, and 10 mM potassium phosphate buffer (pH 8). The time course of the reaction was monitored by subjecting 30-μl aliquots of the incubation mixture to the assay of Lizada and Yang (21). The total yields of ACC were 3.5 μmol for the *trans* isomer (Fig. 1) of AdoMet and 0.9 μmol for the *cis* isomer.

Isolation and Purification of ACC from the Incubation Mixtures. The enzyme incubation mixtures were separately freeze-dried and extracted three times with 50 ml of 80% ethanol. The extract was evaporated to dryness and then dissolved in 50% ethanol (0.5 ml), and the ethanolic solution was applied to a 20 × 20 cm analytical TLC plate (Analtech silica gel GF, 250 μm). The plate was developed ascendingly with butanol/acetic acid/water (13:2:5), and the ACC band was scraped from the plate. ACC was extracted from the silica gel with ethanol, the ethanol was evaporated, and the ACC was dissolved in water. The sample was applied to a 5-ml Dowex 50X4-200 (NH_4^+) column, washed with water to remove traces of acetic acid, and eluted with 1 M NH_4OH. The fractions containing ninhydrin-positive material were pooled and freeze-dried to give ACC.

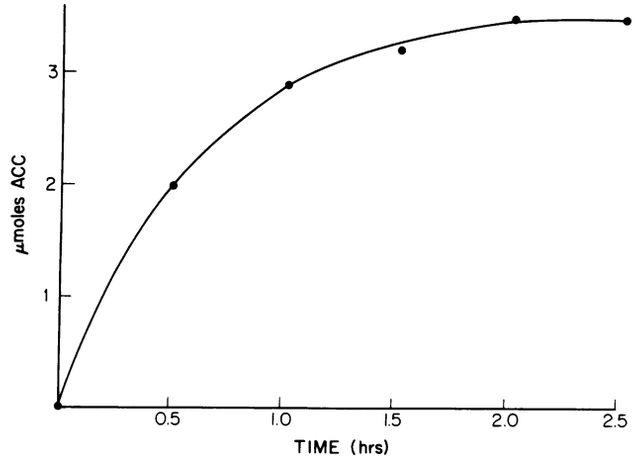

FIG. 1. Time-dependent formation of ACC from *trans*-AdoMet, determined by subjecting 30-μl aliquots of the incubation mixture to the assay procedure of Lizada and Yang (21). Final yield was 3.5 μmol of ACC (85%).

RESULTS

In order to obtain mechanistic information from a stereochemical study of an enzyme, four distinct tasks are necessary: (*i*) the synthesis of stereospecifically labeled substrates of known absolute configuration, (*ii*) enzymatic conversions of these substrates into the enzyme reaction products, (*iii*) analysis of these products to determine the resulting configuration of the stereospecifically labeled center(s) in the product, and (*iv*) interpretation of the results.

The synthesis of the required AdoMet samples, (−)-*S*-adenosyl-L-(3*R**,4*S**)-[3,4-$^2H_2$]methionine and (−)-*S*-adenosyl-L-(3*R**,4*R**)-[3,4-$^2H_2$]-methionine, started, respectively, from *Z*- and *E*-[1,2-$^2H_2$]ethene, which were converted into 1-chloro-2-(methylthio)ethanes via reaction with methanesulfenyl chloride. Reaction of the chlorides with the sodium salt of diethyl acetamidomalonate followed by acid hydrolysis gave the respective racemic methionine (16). Each of the two methionine samples produced consisted of a mixture of four stereoisomers. The enzymatic activation, via AdoMet synthetase, of either of these stereospecifically deuterated methionine mixtures into the corresponding AdoMet samples would have led to the desired species, (−)-*S*-adenosyl-L-(3*R**,4*S**)-[3,4-$^2H_2$]methionine and (−)-*S*-adenosyl-L-(3*R**,4*R**)-[3,4-$^2H_2$]methionine, in which both the sulfonium center and the amino acid center would have possessed the natural configuration, 2*S*, S(*S*) (22, 23). However, the laborious process of obtaining sufficient quantities of AdoMet synthetase and the relatively low yields in this enzymatic reaction forced us to activate the above methionines chemically, thus creating two mixtures, (±)-*S*-adenosyl-DL-(3*S**,4*R**)-[3,4-$^2H_2$]methionine (*cis*-AdoMet) and (±)-*S*-adenosyl-DL-(3*R**,4*R**)-[3,4-$^2H_2$]methionine (*trans*-AdoMet), each containing eight stereoisomers ($2^4 = 16$ total possible). However, it has been shown (24) that ACC synthase utilizes only naturally occurring AdoMet, which contains the L α-amino acid center in the methionine portion and the *S* configuration at the sulfonium center [S(*S*)]. Therefore only two isomers of the eight in each mixture will be transformed into ACC (i.e., those with the "*S*" configuration at both the sulfur center and α-amino acid center: (−)-*S*-adenosyl-L-(3*R**,4*S**)-[3,4-$^2H_2$]methionine and (−)-*S*-adenosyl-L-(3*R**,4*R**)-[3,4-$^2H_2$]methionine). The two stereoisomeric samples of AdoMet (all eight stereoisomers in each mixture) were incubated separately with ACC synthase purified from tomatoes by the method of Acaster and Kende (20). Although the specific activity of the enzyme was quite low and each mixture contained several isomers of AdoMet that are known to be inhibitors (24) of ACC synthase, significant quantities of the natural AdoMet had been converted to ACC after 3 hr. The reaction products were extracted from the lyophilized reaction mixtures with 80% ethanol and purified first by preparative TLC on silica gel and then by ion-exchange chromatography. Because of the unstable nature of AdoMet in solutions at a pH > 5, attempts to reisolate the unreacted, inactive isomers of AdoMet proved futile.

The third task involved the determination of the configuration of the ACC samples produced. The product(s) obtained from the *cis* and *trans* AdoMet samples should be either *cis*- or *trans*-[2,3-$^2H_2$]ACC or, potentially, a monodeuterated ACC if there is any β-hydrogen exchange. Authentic samples of *cis*- and *trans*-[2,3-$^2H_2$]ACC were prepared chemically, following previously reported procedures (17), from the same *E*- and *Z*-[1,2-$^2H_2$]ethene used to prepare the AdoMet samples used in this study. Comparison of the ^{1}H-NMR spectra of the chemically and enzymatically prepared [2,3-$^2H_2$]ACC samples allows the determination of the configuration of the enzymatically prepared products. Although we know that, in ACC, the two hydrogens *cis* to the carboxylate function resonate at δ = 1.14 ppm and the two hydrogens *trans* to the carboxylate function resonate at δ = 0.99 (unpublished results), in the present study it does not matter which is which. Since the chemically derived *cis*-[2,3-$^2H_2$]ACC sample is a racemic mixture at the α position, we have the sum of the two ^{1}H-NMR spectra of the individual isomers (i.e., one isomer with two hydrogens *cis* to the carboxylate and one isomer with two hydrogens *trans* to the carboxylate). Thus the ^{1}H-NMR spectrum of synthetic *cis*-[2,3-$^2H_2$]ACC (Fig. 2) shows singlets at δ = 1.14 ppm and δ = 0.99 ppm, which, when integrated, are in a 1:1 ratio. The ^{1}H-NMR spectrum of the synthetic *trans*-[2,3-$^2H_2$]ACC shows doublets at δ = 1.14 ppm (*J* = 7 Hz, due to the coupling of the hydrogen *cis* to the carboxylate function to the hydrogen *trans* to the carboxylate function) and δ = 0.99 (*J* = 7 Hz), which, when integrated, are in a 1:1 ratio. The ^{1}H-NMR spectrum of the racemic synthetic *trans* isomer is again a composite of the ^{1}H-NMR spectra of the two stereoisomers, which in this case would each give the same spectra. The small amount of undeuterated ethene that contaminates the *Z*-[1,2-$^2H_2$]ethene is processed both chemically and enzymatically to give what appears in the ^{1}H-NMR spectra to be some *cis*-[ACC] contaminating the *trans*-[ACC].

The ^{1}H-NMR spectra of the enzymatic reaction mixtures are also shown in Fig. 2. The ACC products are also racemic at the α position and thus the ^{1}H-NMR spectra will be composites of both isomers. As can be seen by comparison with the spectra of the synthetic samples, the ACC obtained from (−)-*S*-adenosyl-L-(3*R**,4*R**)-[3,4-$^2H_2$]methionine (*trans*-AdoMet) had the *trans* configuration, whereas the ACC obtained from (−)-*S*-adenosyl-L-(3*S**,4*R**)-[3,4-$^2H_2$]-methionine (*cis*-AdoMet) had the *cis* configuration.

In order to interpret the results at the α- and/or the γ-center of the methionine portion of the stereoisomers of AdoMet, we constructed Fig. 3 to predict which ACC would be formed from which isomer of AdoMet depending on the stereochemical events at the α- and γ-positions, without considering racemization or β-participation as possible events. Also shown are the predicted individual ^{1}H-NMR spectra of the two possible ACC species derived from the two enzymatically active isomers of AdoMet in each substrate sample. It should be remembered that the experimentally observed

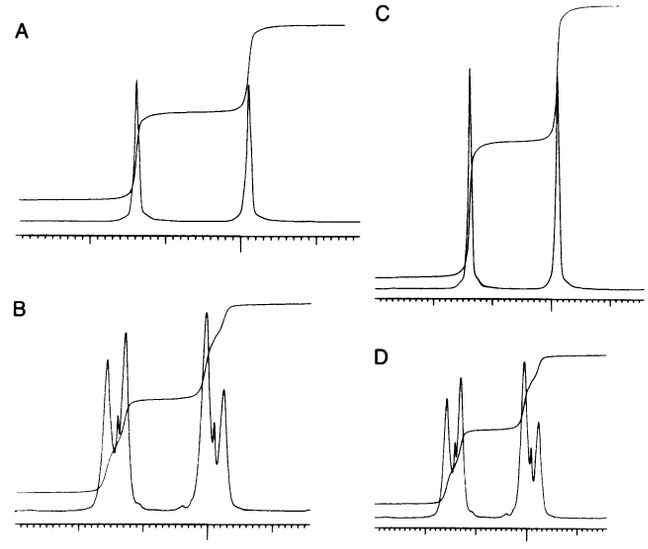

FIG. 2. The 270-MHz ^{1}H-NMR spectra of chemically synthesized *cis*-ACC (*A*), chemically synthesized *trans*-ACC (*B*), enzymatically synthesized *cis*-ACC (*C*), and enzymatically synthesized *trans*-ACC (*D*).

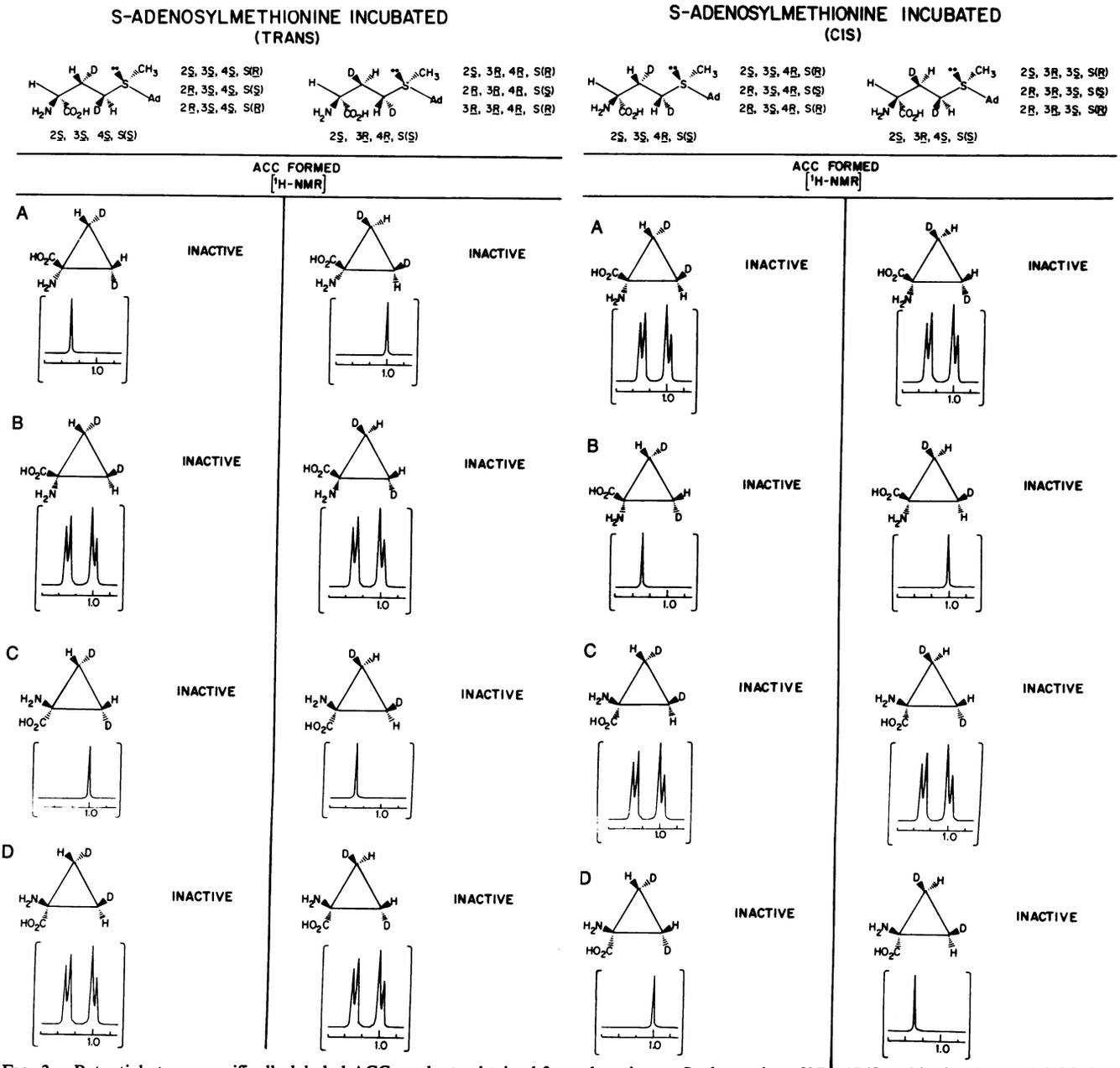

FIG. 3. Potential stereospecifically labeled ACC products obtained from the mixture *S*-adenosyl-DL-[3*R**,4*R**]methionine (*trans*-AdoMet) (*Left*) and from the mixture *S*-adenosyl-DL-[3*S**,4*R**]methionine (*cis*-AdoMet) (*Right*) by various combinations of stereochemical events at the α- and γ-positions such as α-retention and γ-retention (*A*); α-retention and γ-inversion (*B*); α-inversion and γ-retention (*C*); and α-inversion and γ-inversion (*D*). This scheme does not consider β-participation or racemization as possible events. For each mixture, the two enzymatically active AdoMet substrates are depicted, and the other six isomers are only listed. The 270-MHz ^{1}H-NMR spectrum of each ACC product is shown below the structure of the product. Ad, 5′-deoxyadenosyl; D, deuterium.

^{1}H-NMR spectra will be composites of the two individual spectra shown in each row of Fig. 3 *Left* or *Right*. If a *cis*- or *trans*-ACC is obtained from a given AdoMet reaction mixture, the ACC could have been formed by either of two distinct stereochemical pathways. However, the events at the γ carbon will be the same in both stereochemical pathways. This leaves only the stereochemistry of the events at the α carbon unknown.

DISCUSSION

The participation of the β-hydrogen(s) of AdoMet in the enzymatic conversion of AdoMet to ACC appears unlikely because the ^{1}H-NMR spectra of the ACC samples obtained from the enzymatic reactions were identical to the spectra of the ACC samples chemically synthesized. The two enzymatically active AdoMet species in each incubation mixture contained a deuterium in either the 3*R* or 3*S* β-position. Therefore, the abstraction of a β-hydrogen (without isotopic discrimination between the two stereoisomers in each of the incubation mixtures) and subsequent exchange (either partial or total) of deuterons with protons of the medium would have resulted in the loss of deuterium from the β-position and the formation of an ACC species in which the integration of the peaks at $\delta = 1.14$ ppm and $\delta = 0.99$ ppm would not have been 1:1. The spectral pattern would also have been quite different. Since the ratio of the two hydrogen signals in the ^{1}H-NMR spectra of the enzymatically produced ACC samples is exactly 1:1, each substrate mixture of isomers with deuterium in the *pro*-3*R* and *pro*-3*S* position would have had

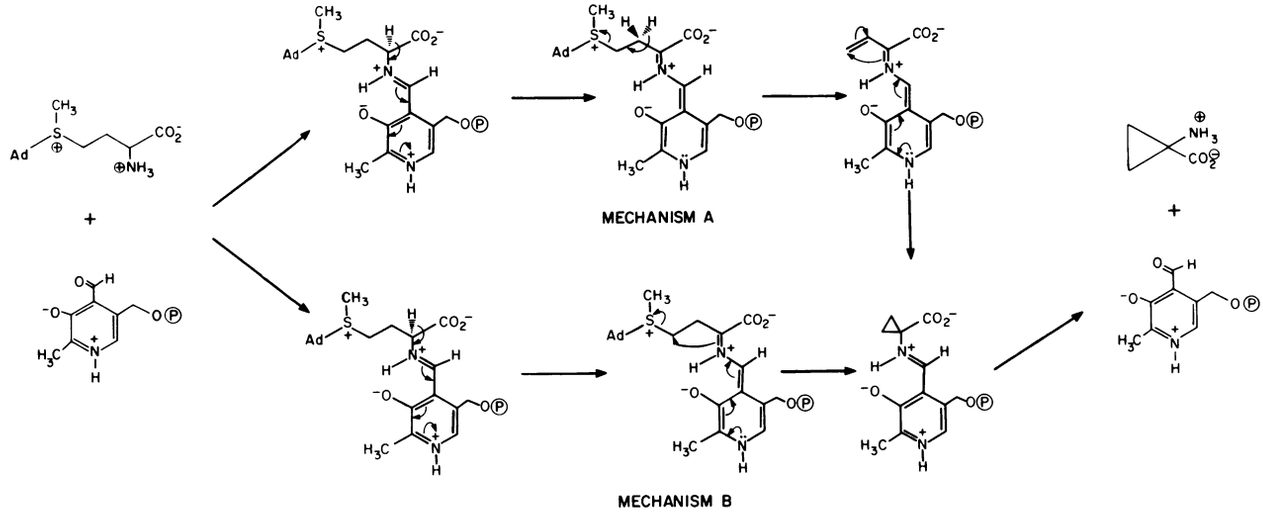

FIG. 4. Two potential mechanisms for the pyridoxal-*P*-catalyzed formation of ACC from AdoMet.

to react at equal rates. If there were hydrogen abstraction at the β carbon, it would be likely to involve an isotope effect, resulting in greater conversion of the isomer carrying a normal hydrogen in the position from which the hydrogen is abstracted. Hence the ratio of the two proton signals in the product would again be different from 1:1. The only process consistent with our findings, which would involve β-hydrogen (deuterium) abstraction, would have necessitated the removal of a β-hydrogen with no isotopic discrimination, followed by 100% return of the removed hydrogen (or deuterium) again with no isotope effect. Since this is highly unlikely, the most logical explanation of the data is that there is no β-hydrogen participation.

From the ^{1}H-NMR spectra of the enzymatically produced ACC samples, one can see that (−)-*S*-adenosyl-L-(3*R**,4*R**)-[3,4-$^2H_2$]methionine (*trans*-AdoMet) gave *trans*-ACC and (−)-*S*-adenosyl-L-(3*S**,4*R**)-[3,4-$^2H_2$]methionine (*cis*-AdoMet) gave *cis*-ACC. Based on Fig. 3, this is consistent with α-inversion,γ-inversion or α-retention,γ-inversion. Although the stereochemical events at the α carbon consistent with the experimental results are ambiguous, the results clearly demonstrate that the stereochemical events at the γ carbon involve overall inversion of configuration. Such an S_N2 mechanism was suggested by Yang and coworkers (3, 8), albeit their prediction was not based on experimental evidence.

From our experimental evidence, we conclude that the reaction involves inversion of configuration at the γ carbon and that the reaction does not involve exchange of β-hydrogens with the solvent. These two results suggest that (*i*) the reaction proceeds by direct displacement of the 5′-methylthio-5′-deoxyadenosine moiety at the γ carbon by an α-carbon carbanion (see Fig. 4, mechanism B) and (*ii*) the reaction does not involve a vinylglycine or similar intermediate (see Fig. 4, mechanism A), unless such an intermediate is formed and further transformed (*a*) with 100% internal hydrogen return and (*b*) without any significant isotope effect in the abstraction of a β-hydrogen. This apparent lack of β-participation and direct γ-displacement makes the reaction mechanism of ACC synthase unique among the pyridoxal-*P*-mediated γ-replacement reactions studied to date.

This work was supported by the Public Health Service through Research Grant GM30097 at the University of Michigan and by the United States Department of Energy under Contract DE-AC02-76ERD-1338 at Michigan State University.

1. Perkin, W. H., Jr. (1884) *Ber. Dtsch. Chem. Ges.* **17**, 54–59.
2. Burroughs, L. F. (1957) *Nature (London)* **179**, 360–361.
3. Adams, D. O. & Yang, S. F. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 170–174.
4. Abeles, F. B. (1973) *Ethylene in Plant Biochemistry* (Academic, New York).
5. Cantoni, G. L. (1952) *J. Am. Chem. Soc.* **74**, 2942–2943.
6. Cameron, A. C., Fenton, C. A. L., Yu, Y., Adams, D. O. & Yang, S. F. (1979) *HortScience* **14**, 178–180.
7. Boller, T., Herner, R. C. & Kende, H. (1979) *Planta* **145**, 293–303.
8. Yu, Y.-B., Adams, D. O. & Yang, S. F. (1979) *Arch. Biochem. Biophys.* **198**, 280–286.
9. Braunstein, A. E. (1963) in *Proceedings of the 1st International Union of Biochemistry Symposium, Rome, October 1962*, eds. Snell, E. E., Fasella, P., Braunstein, A. E. & Rossi-Fanelli, A. (Pergamon, Oxford), p. 581.
10. Floss, H. G. & Vederas, J. C. (1982) *New Comprehensive Biochemistry*, ed. Tamm, C. (Elsevier Biomedical, Amsterdam), Vol. 3, pp. 161–199.
11. Walsh, C. T. (1979) *Enzymatic Reaction Mechanisms* (Freeman, San Francisco), pp. 823–825.
12. Chang, M. N. T. & Walsh, C. T. (1981) *J. Am. Chem. Soc.* **102**, 7638–7370.
13. Chang, M. N. T. & Walsh, C. T. (1981) *J. Am. Chem. Soc.* **103**, 4921–4927.
14. Liu, H.-W., Auchus, R. & Walsh, C. T. (1984) *J. Am. Chem. Soc.* **106**, 5335–5348.
15. Baldwin, J. E. (1976) *J. Chem. Soc. Chem. Commun.*, 734–736.
16. Billington, D. C. & Golding, B. T. (1982) *J. Chem. Soc. Perkin Trans.* **1**, 1283–1290.
17. Ramalingam, K., Kalvin, D. & Woodard, R. W. (1984) *J. of Labelled Comp. Radiopharm.* **21**, 833–841.
18. Kikugawa, K. & Ichino, M. (1971) *Tetrahedron Lett.*, 87–90.
19. Boller, T. (1984) in *Ethylene: Biochemical, Physiological and Applied Aspects*, eds. Fuchs, Y. & Chalutz, E. (Nijhoff/Junk, The Hague, The Netherlands), pp. 87–88.
20. Acaster, M. A. & Kende, H. (1983) *Plant Physiol.* **72**, 139–145.
21. Lizada, M. C. C. & Yang, S. F. (1979) *Anal. Biochem.* **100**, 140–145.
22. Chiang, P. K. & Cantoni, G. L. (1977) *J. Biol. Chem.* **252**, 4506–4513.
23. Cornforth, J. W., Reichard, S. A., Talalay, P., Carrell, H. L. & Glusker, J. P. (1977) *J. Am. Chem. Soc.* **99**, 7292–7300.
24. Oskouee, S. K., Jones, J. P. & Woodard, R. W. (1984) *Biochem. Biophys. Res. Commun.* **121**, 181–187.