Plant Physiol. (1984) 75, 192–195
0032-0889/84/75/0192/04/$01.00/0

# Ethylene-Enhanced 1-Aminocyclopropane-1-carboxylic Acid Synthase Activity in Ripening Apples[1]

Received for publication July 26, 1983 and in revised form November 30, 1983

GEBHARD BUFLER*
*Institut für Obstbau (370), Universität Hohenheim, 7000 Stuttgart 70, Federal Republic of Germany*

## ABSTRACT

**Apples (*Malus sylvestris* Mill, cv Golden Delicious) were treated before harvest with aminoethoxyvinylglycine (AVG). AVG is presumed to reversibly inhibit 1-aminocyclopropane-1-carboxylic acid (ACC) activity, but not the formation of ACC synthase. AVG treatment effectively blocked initiation of autocatalytic ethylene production and ripening of harvested apples. Exogenous ethylene induced extractable ACC synthase activity and ripening in AVG-treated apples. Removal of exogenous ethylene caused a rapid decline in ACC synthase activity and in $CO_2$ production. The results with ripened, AVG-treated apples indicate (a) a dose-response relationship between ethylene and enhancement of ACC synthase activity with a half-maximal response at approximately 0.8 μl/l ethylene; (b) reversal of ethylene-enhanced ACC synthase activity by $CO_2$; (c) enhancement of ACC synthase activity by the ethylene-activity analog propylene.**

**Induction of ACC synthase activity, autocatalytic ethylene production, and ripening of preclimacteric apples not treated with AVG were delayed by 6 and 10% $CO_2$, but not by 1.25% $CO_2$. However, each of these $CO_2$ concentrations reduced the rate of increase of ACC synthase activity.**

The onset of ripening in climacteric fruits is marked by a burst of ethylene production (1, 14). Exogenous ethylene can induce ripening and, concomitantly, endogenous ethylene production (1, 14). Once induced, endogenous ethylene stimulates its own biosynthesis, a process called autocatalytic ethylene production (1, 14).

The establishment of the path of ethylene biosynthesis, SAM[2] → ACC → $C_2H_4$ (2), led to investigations on the role ethylene plays in its own biosynthesis. Considering only the autocatalytic effects, it was demonstrated that exogenous $C_2H_4$ stimulates both the formation of ACC and the conversion of applied ACC to $C_2H_4$ in leaf tissue (17). In wounded tissue of preclimacteric cantaloupe, conversion of ACC to $C_2H_4$ can also be enhanced by exogenous $C_2H_4$ (9). The formation of ACC from SAM is catalyzed by ACC synthase (4, 21), a rate controlling enzyme in ethylene biosynthesis (19). In preclimacteric apples stored under hypobaric conditions, both exogenous $C_2H_4$ and the ethylene activity analog $C_3H_6$ induce and enhance ACC synthase activity, thereby inducing the formation of ACC (6).

The present investigation attempts to characterize the action of ethylene on ACC synthase activity in intact apples on the basis of three experiments (7): (a) dose-response relationship, (b) effect of $CO_2$, a known inhibitor of ethylene action, and (c) effect of the ethylene activity analog propylene.

However, a major obstacle in studying ethylene action in plant tissue is the presence of endogenous ethylene. Therefore, in order to exclude endogenously produced ethylene, in some of these experiments apples were treated before harvest with AVG, an inhibitor of ethylene biosynthesis (11). When sprayed on apple trees before harvest, AVG inhibits ethylene production and delays fruit ripening (3). Ethylene treatment of AVG-infiltrated pear fruits resulted in a climacteric rise in respiration, softening, and color change, while endogenous ethylene production was still inhibited (15).

Inhibition of ethylene production by AVG is mediated by inhibition of ACC synthase activity (2, 4, 21). Since inhibition of ACC synthase activity by AVG is reversible *in vitro* (4), it is presumed that removal of AVG from the tissue extract should allow estimation of the effect of exogenous ethylene on the content of ACC synthase activity in AVG-treated apples.

## MATERIALS AND METHODS

**Plant Material.** Preclimacteric apples (*Malus sylvestris* Mill, cv Golden Delicious) were stored immediately after harvest at 4°C and at 6.6 kPa reduced pressure. This prevents ripening and ethylene production for at least 4 months (6). Therefore, during this time period, preclimacteric apples were available for experiments. Apples, sprayed with AVG 4 weeks (500 mg active ingredient/l) and 3 weeks (200 mg/l) before harvest, were harvested on the same day as nontreated fruits and stored immediately after harvest at 4°C in a stream of moist air. AVG-treated apples did not ripen or produce ethylene under these conditions for at least 2 months. Therefore, AVG-treated apples were considered preclimacteric during this time period and used within 2 months after harvest for experiments.

**Chemicals.** AVG, in the form of a wettable powder with 20% active ingredient, was obtained from Dr. R. Maag AG (Dielsdorf, Switzerland). SAM and pyridoxal phosphate were purchased from Boehringer, ACC from Calbiochem, Tricine and DTE from Serva, and Sephadex G-50 from Pharmacia Fine Chemicals.

**Gas Treatments.** All experiments were carried out in temperature-regulated chambers at 25°C. AVG-treated apples were equilibrated at 25°C for at least 36 h before gas treatments. Apples, previously stored at 6.6 kPa and 4°C, were subjected to gas treatments immediately after transfer to atmospheric pressure and 25°C. In general, uniformly sized apples were placed in desiccators (21 L) connected to a gas flow system. Ethylene was premixed with $N_2$ in pressure cylinders to give concentrations of 10,000 μl/l and 100 μl/l. These gas mixtures were mixed with air in gas-mixing pumps (Wösthoff oHG, Bochum, W. Germany), which then delivered ethylene concentrations of approximately 100, 10, 1, and 0.1 μl/l to the desiccators. The resulting $O_2$ concentrations did not differ significantly from that in air. After mixing, the gas streams were humidified and the flow rates

[1] Supported by Deutsche Forschungsgemeinschaft.

[2] Abbreviations: SAM, *S*-adenosylmethionine; ACC, 1-aminocyclopropane-1-carboxylic acid; AVG, aminoethoxyvinylglycine(2-amino-4[2-aminoethoxy]-*trans*-3-butenoic acid).

adjusted to 40 l/h before reaching the fruits in the desiccators. Basically, the same system was used when apples were treated with mixtures of $C_2H_4$ and $CO_2$, except that a known concentration of $C_2H_4$ was premixed with air and then mixed by gas-mixing pumps with $CO_2$. When apples were treated only with different concentrations of $CO_2$, $CO_2$ was mixed with air by gas-mixing pumps. Dilution of $O_2$ concentration caused by addition of $CO_2$ was not corrected. Air was passed through vermiculite coated with $KMnO_4$ to deliver 'ethylene-free' air. Treatment of AVG apples with propylene (or ethylene) was performed in a static system. Four apples were enclosed in a desiccator (21 L) and a known amount of propylene (ethylene) was added. A solution of mercury perchlorate was present in the propylene-free or ethylene-free treatment to trap ethylene produced by the apples. NaOH (0.1 N) was present in each treatment to trap $CO_2$. Propylene (ethylene) treatment was completed after 24 h. In all experiments, concentrations of $C_2H_4$, $C_3H_6$, and $CO_2$ supplied to the apples were verified by GC. Ethylene and $CO_2$ production of apples were calculated from gas concentrations in the air stream leaving the desiccators.

**Sampling Procedure.** At each sampling time, four fruits per treatment were removed from the desiccators. One tissue segment (1 g) was cut from the equatorial region of a fruit and combined with segments from three other fruits. Four segments (4 g) from four apples were used for each extraction. Extraction was repeated with tissue segments frozen in liquid $N_2$, lyophilized, and stored at −20°C until required. The absolute amounts of ACC synthase were slightly lower in lyophilized tissue. Therefore, only results from fresh tissue are presented. Each experiment was repeated once, yielding similar results.

**Fruit Firmness.** A pressure tester (Effigi penetrometer, Garibaldi, Italy) with a 10-mm plunger was used to measure the force required to puncture the peeled flesh of the fruit.

**Extraction of ACC and ACC Synthase.** ACC synthase was extracted as described previously (6). The extraction medium for ACC synthase and ACC contained 100 mM Tricine-KOH (pH 8.5), 4 mM DTE, 5 μM pyridoxal phosphate and 0.2% (v/v) Triton X-100. Four grams of fresh tissue were homogenized with 8 ml of extraction medium, using an Ultra Turrax Homogenizer. The homogenate was centrifuged for 15 min at 25,000*g*, the pellet rehomogenized with 4 ml of extraction medium, centrifuged again as above, and the two supernatants combined. An aliquot of the combined supernatant was used for routine determinations of ACC according to the method of Lizada and Yang (12). For the enzyme extraction, 2 ml of the supernatant were purified on a Sephadex G-50 column (14 × 1 cm), previously equilibrated with 5 mM Tricine-KOH (pH 8.0) containing 0.1 mM DTE, and 1 μM pyridoxal phosphate. The protein fraction was collected and used in the enzyme assay. When ACC synthase was extracted from AVG-treated apples, the concentration of pyridoxal phosphate was 10 μM in the extraction medium and 5 μM in the equilibration medium for gel filtration (9). All steps in the enzyme extraction were carried out at 4°C.

**Assay of ACC Synthase.** ACC synthase activity was determined according to Boller *et al.* (4). The reaction mixture consisted of 0.4 ml of protein fraction, 0.1 ml of 100 mM Tricine-KOH (pH 8.0), and 0.1 ml of 65 μM SAM. SAM was omitted from the blank samples. The concentration of pyridoxal phosphate in the assay mixture was adjusted to 15 μM in the case of AVG-treated apples, instead of 1 μM in the case of untreated apples. The amount of enzyme used in the assay was in the linear range of activity. Protein content of the assay mixture was in the range of 40 to 80 μg. After incubation of the reaction mixture for 1 h at 30°C, the ACC formed was assayed by the method of Lizada and Yang (12). Addition of 30 μM AVG to the assay mixture inhibited ACC synthase activity completely.

**Protein Determination.** Protein concentration was determined by the dye-binding method of Bradford (5).

## RESULTS

**Effects of Ethylene on Ripening and ACC Synthase Activity in AVG-Treated Apples.** Ethylene treatment caused ripening of AVG-treated apples, expressed as an increase in softening (Fig. 1A), $CO_2$ production (Fig. 1B), and a change in peel color from green to yellow. Production of $CO_2$ and rate of softening were positively related to the concentration of applied $C_2H_4$ (Fig. 1, A and B), as was previously reported by Halder-Doll (8). Approximately 10 μl/l $C_2H_4$ was a saturating dose. In addition to fruit ripening, ACC synthase was induced by ethylene in AVG-treated apples (Fig. 1C); 1 μl/l $C_2H_4$ caused occasional activity, whereas

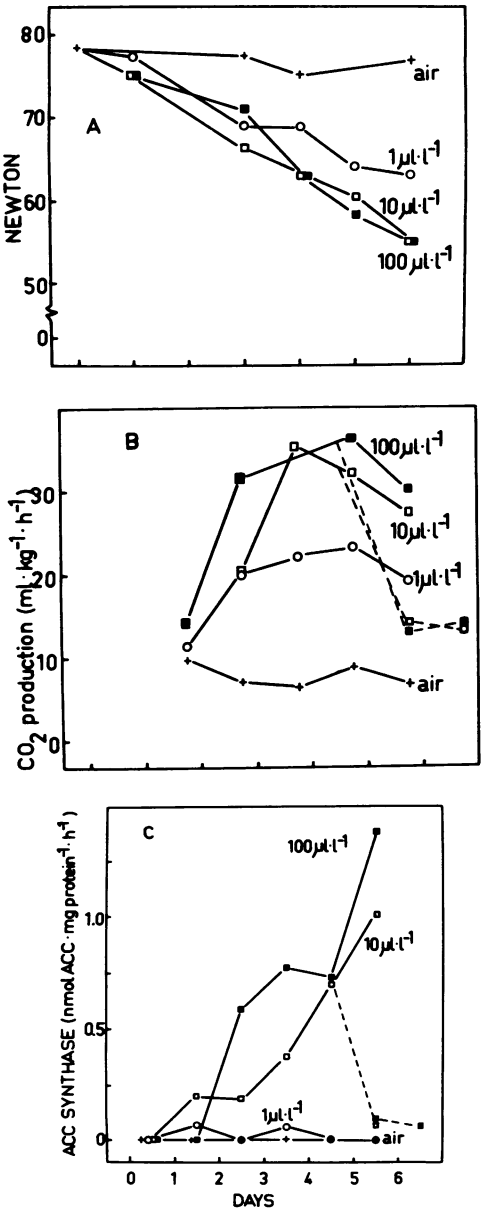


FIG. 1. Effect of different concentrations of ethylene on firmness (A), $CO_2$ production (B), and ACC synthase activity (C) of AVG-treated apples. After equilibration at 25°C, apples were treated with ethylene-free air (+), 1 μl/l (○), 10 μl/l (□), or 100 μl/l (■) $C_2H_4$. (---), $CO_2$ production (B) or ACC synthase activity (C) of apples after removal from ethylene atmosphere. One Newton = 0.225 pound force.

10 μl/l and 100 μl/l increased ACC synthase activity readily in a step-like manner. The ACC content of ethylene-treated apples did not change. It remained on a preclimacteric level below 0.1 nmol/g fresh weight; *i.e.* ACC synthase was active *in vitro* but not *in vivo* in the presence of AVG. No ACC synthase activity was detectable in AVG-treated apples placed in ethylene-free air. Transferring apples from ethylene atmosphere to ethylene-free air caused ACC synthase activity to decline almost to nil in 24 h (Fig. 1C). It should also be noted that a decline in $CO_2$ production followed the removal of ethylene from AVG-treated apples (Fig. 1B).

**Dose-Response Relationship between Ethylene and ACC Synthase Activity in AVG-Treated Apples.** Since induction of ACC synthase activity by low ethylene concentration was erratic in preclimacteric apples (Fig. 1C), the relationship between ethylene and ACC synthase was studied in postclimacteric apples. For this purpose, AVG-treated apples were ripened for 4 d with 100 μl/l $C_2H_4$ until maximum $CO_2$ production and then followed by ventilation with ethylene-free air for another 4 d, with repeated evacuations to remove the remaining traces of ethylene from the fruits. When the apples were free of ACC synthase activity, treatments with different concentrations of ethylene were started. After 8 h, ACC synthase activity was increased in apples at all ethylene concentrations (Fig. 2). After 24 h, dependency of ACC synthase activity on ethylene concentration became obvious. Plotting ACC synthase activity against logarithmic concentrations of ethylene (from Fig. 2; 48 h), yields a sigmoid curve, suggesting a half-saturating dose of ethylene at approximately 0.8 μl/l. The application of a Lineweaver-Burk plot gives a straight line, dissecting the abscissa at 0.8 μl/l $C_2H_4$.

**Effect of $CO_2$ on ACC Synthase of AVG-Treated Apples in the Presence of Exogenous Ethylene.** AVG-treated apples, used in this experiment, were ripened as described above. Apples were then ventilated for 48 h with a certain concentration of $C_2H_4$, until $CO_2$ treatment was started in combination with the ethylene treatment. After 12 h $CO_2$ treatment, no significant difference between the various treatments was observed (data not shown). However, after 36 h $CO_2$ treatment, $CO_2$ reversed ethylene-enhanced ACC synthase activity in the presence of 2 μl/l $C_2H_4$, depending on the concentration of $CO_2$ (Table I). In the presence of 200 μl/l $C_2H_4$, 10% $CO_2$ had a reversing effect, whereas the lower concentrations of $CO_2$ had no effect on ACC synthase activity.

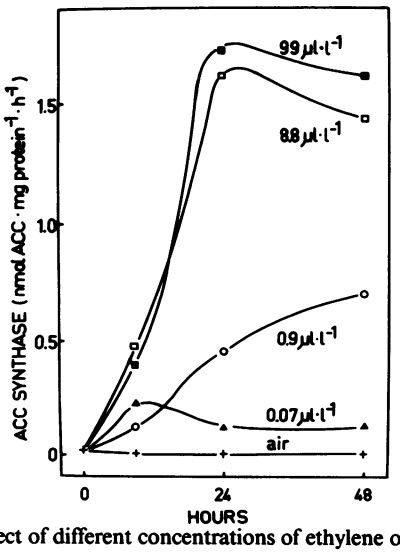


FIG. 2. Effect of different concentrations of ethylene on the development of ACC synthase activity in AVG-treated apples after ripening. Apples were treated with 100 μl/l $C_2H_4$ for 4 d, then ventilated with ethylene-free air for another 4 d. Thereafter (time, 0 h), apples were treated with ethylene-free air (+), 0.07 μl/l (Δ), 0.9 μl/l (O), 8.8 μl/l (□), or 99 μl/l (■) $C_2H_4$.

Table I. *Effect of $CO_2$ on Ethylene-Enhanced ACC Synthase Activity*

AVG-treated apples were ripened for 4 d with 20 μl/l $C_2H_4$. Thereafter, $C_2H_4$ was replaced by ethylene-free air for another 4 d, followed by ventilation with 2 or 200 μl/l $C_2H_4$ for 48 h. Different concentrations of $CO_2$ were then mixed with the ethylene-containing gas stream. Relative activities of ACC synthase after 36 h of $CO_2$ treatment are presented. ACC synthase activity of ethylene treatment was set at 100. Absolute amounts of ACC synthase activity were 0.64 nmol ACC/mg protein·h for 2 μl/l $C_2H_4$ and 2.17 nmol ACC/mg protein·h for 200 μl/l $C_2H_4$.

| Treatment | Relative ACC Synthase Activity |
|---|---|
| 2 μl/l $C_2H_4$ | 100 |
| 2 μl/l $C_2H_4$ + 1% $CO_2$ | 67 |
| 2 μl/l $C_2H_4$ + 5% $CO_2$ | 55 |
| 2 μl/l $C_2H_4$ + 10% $CO_2$ | 33 |
| 200 μl/l $C_2H_4$ | 100 |
| 200 μl/l $C_2H_4$ + 1% $CO_2$ | 100 |
| 200 μl/l $C_2H_4$ + 5% $CO_2$ | 100 |
| 200 μl/l $C_2H_4$ + 10% $CO_2$ | 55 |

**Effect of $CO_2$ on Induction and Development of ACC Synthase Activity in Preclimacteric Apples Not Treated with AVG.** Apples, previously stored at reduced atmospheric pressure and 4°C, were used. No exogenous $C_2H_4$ was applied during the course of the experiment. ACC synthase activity was not detectable in apples removed immediately from hypobaric storage (day 0; Fig. 3A). Fruits kept in air developed ACC synthase activity at the same time as fruits kept in 1.25% $CO_2$. However, after 5.5 days the activity of ACC synthase in apples kept in 1.25% $CO_2$ was only 40% of the activity of apples kept in air, although ethylene production amounted to 85% of that in air (Fig. 3B). Ten and 6% $CO_2$ delayed the induction of ACC synthase by 1 d and slowed the increase in its activity. The burst of ethylene production (Fig. 3B) was also delayed by 6 and 10% $CO_2$.

**Effect of Propylene on ACC Synthase Activity of AVG-Treated Apples.** The effect of 5 μl/l $C_2H_4$ was compared with the effect of 650 μl/l $C_3H_6$. AVG-treated, ripened apples, as described above, were used in this experiment. Propylene (650 μl/l) stimulated ACC synthase activity to a similar extent as 5 μl/l $C_2H_4$ (1.54 and 1.65 nmol ACC/mg protein/h, respectively).

## DISCUSSION

Apparently two sites in ethylene biosynthesis of climacteric fruits are enhanced by ethylene: (a) formation of ACC *via* stimulation of ACC synthase activity (6; Fig. 2), and (b) formation of ethylene from ACC (9). Many ethylene-mediated processes need the continuous presence of $C_2H_4$ for full expression (1). Removal of ethylene from excised cantaloupe tissue resulted in reduced *in vivo* activity of the ethylene-forming enzyme (9). Removal of $C_2H_4$ from AVG-treated apples caused ACC synthase activity to decline almost to nil in 24 h (Fig. 1C). Thus, autocatalytic ethylene production in ripening fruits may be enhanced and sustained by continuous stimulation of ACC synthase activity and ethylene-forming enzyme activity by means of endogenously produced ethylene.

In ethylene biosynthesis of climacteric fruits, at least ethylene-enhanced ACC synthase activity can be reversed by $CO_2$ (Table I). $CO_2$, at 6 and 10%, also delayed induction of ACC synthase activity (Fig. 3A) and onset of autocatalytic ethylene production (Fig. 3B) of preclimacteric apples. The effects of $CO_2$ in delaying the onset of ripening and the onset of autocatalytic ethylene production, and in reducing ethylene production have long been known (10, 20). It appears that these effects of $CO_2$ can now be ascribed, at least in part, to the inhibition of development of ACC synthase by $CO_2$.

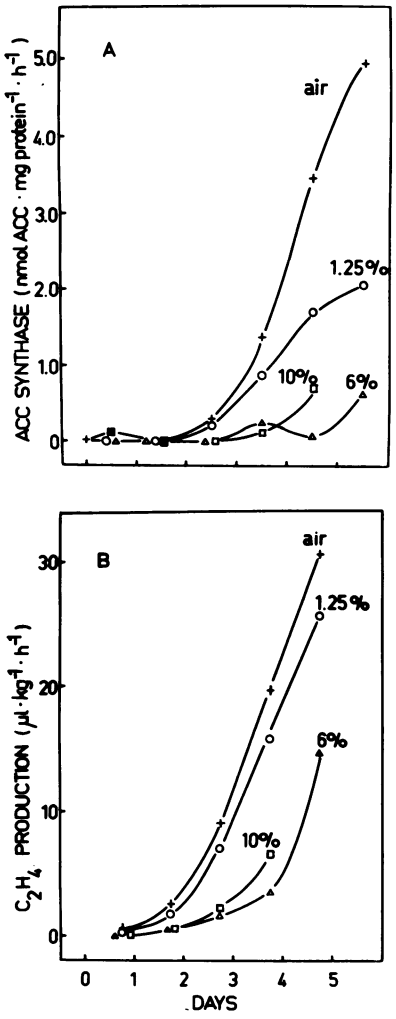


FIG. 3. Effect of different concentrations of $CO_2$ on induction and development of ACC synthase activity (A) and ethylene production (B) in preclimacteric apples. Apples were transferred from hypobaric storage to normal pressure and 25°C and immediately treated with air (+), 1.25% (○), 6% (△), or 10% (□) $CO_2$.

Most of the reported (1) ethylene-mediated processes require 0.01 µl/l $C_2H_4$ for a threshold effect, 0.1 µl/l for a half-maximal effect, and 10 µl/l for a saturating effect. Stimulation of ACC synthase activity in AVG-treated apples, however, requires higher concentrations of $C_2H_4$, *e.g.* 0.8 µl/l for half-maximal stimulation. This discrepancy could be explained on the basis of reduced ethylene action due to the presence of high $CO_2$ (Table I), regardless of whether the mechanism will prove to be competitive (7) or indirect (18). The internal atmosphere of AVG-treated apples stored in ethylene-free air contained 1.7 ± 0.2% $CO_2$ (mean ± SE of five apples; apples were immersed in water and gas was sampled from the core with a syringe). According to the enhancement of $CO_2$ production by exogenous $C_2H_4$ (Fig. 1B), ethylene-treated AVG apples must have contained $CO_2$ in excess of 2%, which might have a reversing effect on ethylene-enhanced ACC synthase activity (cf. Table I). However, this reversing effect by $CO_2$ (up to 5%) can apparently be overcome by an increased ethylene concentration (Table I). Therefore, the necessity for increased amounts of ethylene in order to stimulate ACC synthase activity in apples, is probably due to the presence of relatively high concentrations of internal $CO_2$. This would corroborate Burg and Burg (7) who stated, that "it is probably this endogenous $CO_2$ which raises the threshold for ethylene action in fruits to a slightly higher level than in vegetative tissue."

Climacteric respiration is usually regarded as being unaffected by ethylene concentration, probably because the endogenous ethylene confounded the action of exogenous ethylene (14). Blockage of endogenous ethylene production by AVG however, reveals that $CO_2$ production is positively correlated to the applied concentration of $C_2H_4$ (8; Fig. 1B). Moreover, similar to ACC synthase activity, elevated $CO_2$ production needs the continuous presence of $C_2H_4$ (Fig. 1B). In this respect, AVG-treated fruits behave like nonclimacteric fruits (14). The rate of softening also depends on ethylene concentration (8; Fig. 1A). Ethylene-enhanced ACC synthase activity and ethylene-enhanced ripening of AVG-treated apples, therefore, suggest an indispensable function of ethylene after the initiation of ripening, as proposed earlier by Quazi and Freebairn (16).

A critical assessment of the possible side effects of AVG in apples, with respect to ACC synthase activity, is opportune. It was generally noticed that extractable ACC synthase activity from AVG-treated apples never exceeded 50% of the activity extractable from nontreated apples (cf. Figs. 1C and 3A). One possible explanation for this difference could be an inhibition of the formation of ACC synthase caused by AVG. AVG at concentrations of 1 mM and above can inhibit protein synthesis in plant tissue (13). However, AVG sprayed on apples before harvest with twice the concentration used in the present investigation, resulted in less than 0.01 mM AVG in these apples at harvest (8). In addition, the protein content of ripe, AVG-treated apples was not significantly different from the protein content of ripe, nontreated apples (8). Nevertheless, based only on these data, inhibition of the synthesis of ACC synthase by AVG cannot be ruled out. Alternatively, failure to completely remove AVG during extraction of ACC synthase activity could also account for the lower activity in AVG-treated apples.

*Acknowledgments*—Thanks are due to Drs. H. Halder-Doll and F. Bangerth for critical discussion; Dr. R. Maag AG for providing AVG; and to Dr. S. Waters for reading the English manuscript.

## LITERATURE CITED

1. ABELES FB 1973 Ethylene in Plant Biology. Academic Press, New York
2. ADAMS DO, SF YANG 1979 Ethylene biosynthesis: Identification of 1-aminocyclopropane-1-carboxylic acid as an intermediate in the conversion of methionine to ethylene. Proc Natl Acad Sci USA 76: 170–174
3. BANGERTH F 1978 The effect of substituted amino-acid on ethylene biosynthesis, respiration, ripening and preharvest drop of apple fruits. J Am Soc Hortic Sci 103: 401–404
4. BOLLER T, RC HERNER, H KENDE 1979 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-dye binding. Anal Biochem 72: 248–254
6. BUFLER G, F BANGERTH 1983 Effects of propylene and oxygen on the ethylene-producing system of apples. Physiol Plant 58: 486–492
7. BURG SP, EA BURG 1967 Molecular requirements for the biological activity of ethylene. Plant Physiol 42: 144–152
8. HALDER-DOLL H 1982 Auswirkungen des Ethylensyntheseinhibitors Aminoethoxyvinylglycin auf verschiedene praktische und physiologische Parameter der Reife von Apfelfrüchten. PhD thesis. Universität Hohenheim
9. HOFFMAN NE, SF YANG 1982 Enhancement of wound-induced ethylene synthesis by ethylene treatment in preclimacteric cantaloupe. Plant Physiol 69: 317–322
10. KIDD F, C WEST 1934 The influence of the composition of the atmosphere upon the incidence of the climacteric in apples. Gt Brit Dept Sci Ind Res Rept Food Invest Bd 1933: 51–57
11. LIEBERMAN M 1979 Biosynthesis and action of ethylene. Annu Rev Plant Physiol 30: 533–591
12. LIZADA MCC, SF YANG 1979 A simple and sensitive assay for 1-aminocyclopropane-1-carboxylic acid. Anal Biochem 100: 140–145
13. MATTOO AK, JD ANDERSON, E CHALUTZ, M LIEBERMAN 1979 Influence of enol ether amino acids, inhibitors of ethylene biosynthesis, on aminoacyl transfer RNA synthetases and protein synthesis. Plant Physiol 64: 289–292
14. MCGLASSON WB, DL WADE, J ADATO 1978 Phytohormones and fruit ripening. *In* DS Letham, PB Goodwin, TJV Higgins, eds, Phytohormones and Related Compounds—A Comprehensive Treatise, Vol II. Elsevier, Amsterdam, pp 447–493
15. NESS PJ, RJ ROMANI 1980 Effects of aminoethoxyvinylglycine and counteref-fects of ethylene on ripening of Bartlett pear fruits. Plant Physiol 65: 372–376
16. QUAZI MH, HT FREEBAIRN 1970 The influence of ethylene, oxygen, and carbon dioxide on the ripening of bananas. Bot Gaz 131: 5–14
17. RIOV J, SF YANG 1982 Effects of exogenous ethylene on ethylene production in citrus leaf tissue. Plant Physiol 70: 136–141
18. SISLER EC 1982 Ethylene-binding properties of a Triton X-100 extract of mung bean sprouts. J Plant Growth Regul 1: 211–218
19. YANG SF 1980 Regulation of ethylene biosynthesis. Hort Sci 15: 238–243
20. YOUNG RE, RJ ROMANI, JB BIALE 1962 Carbon dioxide effects on fruit respiration. II. Response of avocados, bananas, and lemons. Plant Physiol 37:416–422
21. YU YB, DO ADAMS, SF YANG 1979 1-Aminocyclopropanecarboxylate synthase, a key enzyme in ethylene biosynthesis. Arch Biochem Biophys 198: 280–286