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Enhancement of Ethylene Formation by Selenoamino Acids[1]

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ABSTRACT

Selenomethionine and selenoethionine enhanced ethylene production in senescing flower tissue of *Ipomoea tricolor* Cav. and in auxin-treated pea (*Pisum sativum L.*) stem sections. This enhancement was fully inhibited by the aminoethoxy analog of rhizobitoxine. Methionine did not have a comparable promotive effect, and ethionine partly inhibited ethylene production. When [^{14}C]methionine was applied to flower or pea stem tissue followed by treatment with unlabeled selenomethionine or selenoethionine, the specific radioactivity of the ethylene evolved was considerably reduced. The dilution of the specific radioactivity of ethylene by selenomethionine, and in pea stem sections also by selenoethionine, was greater than the dilution by nonradioactive methionine at the same concentration. These results indicate that both selenoamino acids serve as precursors of ethylene and that they are converted to ethylene more efficiently than is methionine.

Several *in vitro* model systems have been described in which ethylene is formed from methionine through a reaction involving free radicals (6, 9, 13). In our attempts to elucidate the pathway of ethylene biosynthesis, we investigated whether compounds which are known to quench free radicals inhibited ethylene generation in plant tissues. Among the substances tested were selenoamino acids which have been reported to act as protectants against free-radical attack (12). We found that selenomethionine and selenoethionine greatly enhanced ethylene formation in senescing flower tissue and in pea stem sections treated with IAA. Our results indicate that in these tissues both selenoamino acids are better precursors of ethylene than is methionine.

MATERIALS AND METHODS

Plant Material. Morning glory plants (*Ipomoea tricolor* Cav., cv. Heavenly Blue) were grown as described before (2). Rib segments were prepared from flower buds according to Kende and Hanson (5). The following designations are used to describe the age of the flower tissue: day 0, day of opening and fading of the flower; day −1, 1 day before flower opening; day −2, 2 days before flower opening.

Pea seeds, *Pisum sativum* L., cv. Alaska (Vaughan's Seed Co., Downers Grove, Ill.), were imbibed for 5 hr or overnight in aerated tap water. They were sown in Vermiculite and grown in the dark at 25 C for 6 to 9 days. Stem sections (1.0 cm) were isolated under a green safelight according to Lieberman and Kunishi (7).

Incubation of Plant Material. Rib segments were isolated between 4:00 and 5:00 PM and were placed into 5-cm Petri dishes containing three discs of Whatman No. 1 filter paper and 1.7 ml of distilled H_2O or a solution of the chemical to be tested. The dishes were kept overnight in the same growth chamber where the plants were grown. Between 8:30 and 9:00 AM of the following morning, the rib segments were blotted dry, and batches of 15 segments were transferred to 25-ml Erlenmeyer flasks containing three discs of Whatman No. 1 filter paper and 0.8 ml of either distilled H_2O or the appropriate test solution. Each flask was flushed for 2 min with ethylene-free air, sealed with a serum-vial cap and placed into a darkroom at 27 C.

Pea stem sections were isolated between 4:00 and 6:00 PM, and batches of 12 sections were floated in 6-cm Petri dishes on 2 ml of distilled H_2O or a solution of the compound to be tested. The dishes were kept overnight in the dark at 25 C. The stem sections were removed from the Petri dishes between 8:00 and 9:00 AM the next morning. They were rinsed with distilled H_2O, blotted dry, and transferred to 25-ml Erlenmeyer flasks containing 1 ml of distilled H_2O or the appropriate test solution with or without 0.1 mM IAA. Each flask was flushed with ethylene-free air for 2 min, closed with a serum-vial cap and incubated in darkness at 27 C.

The pH of all test solutions was adjusted with 0.1 N NaOH to 6.5 to 7.0. Each experiment was repeated at least three times with similar results.

Determination of Ethylene Formation. Ethylene production was measured by gas chromatography as described earlier (5).

Determination of Specific Radioactivity of Ethylene. The specific radioactivity of ethylene was determined according to Hanson and Kende (3). Radioactivity was measured with a Packard Tri-Carb liquid scintillation spectrometer, model 3375 (Packard Instruments, Downers Grove, Ill.).

Chemicals. L-[U-^{14}C]Methionine (250–260 mCi/mmol) was purchased from New England Nuclear, D,L-selenomethionine, D,L-selenoethionine, D,L-selenocystine, D,L-ethionine, D-ethionine, L-ethionine, and IAA from Sigma, and L-methionine from Nutritional Biochemical Corp. O-Methylhomoserine and the aminoethoxy analog of rhizobitoxine [L-2-amino-4-(2'-aminoethoxy)-*trans*-3-butenoic acid] were gifts of M. Lieberman (USDA, Agricultural Research Service, Beltsville, Md.).

RESULTS

Effect of Selenoamino Acids on Ethylene Synthesis in Flower Tissue. When rib segments excised from flower buds of *I. tricolor* were incubated continuously from the afternoon of day −1 through the evening of day 0 on solutions containing selenomethionine, ethylene production on day 0 commenced earlier and proceeded at a higher rate than in the control tissue (Fig. 1). The total amount of ethylene produced on day 0 by rib segments treated with selenomethionine was usually three times higher than that produced by rib segments incubated on water. This result was surprising since methionine, the precursor of ethylene in morning glory flower tissue (3), had only a small promotive effect on ethylene synthesis (Fig. 1). For comparative reasons, we also examined the effect of O-methylhomoserine on ethylene production in rib segments. This compound is an analog of methionine

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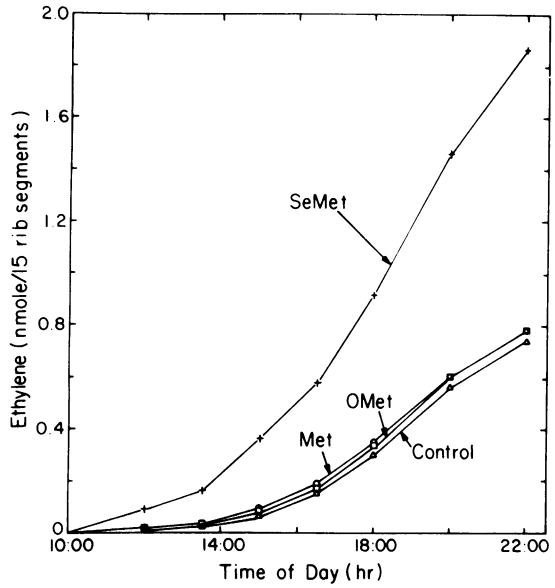

FIG. 1. Effect of methionine analogs on ethylene synthesis in senescing rib segments of *I. tricolor*. Rib segments were excised between 4:00 and 5:00 PM on day −1 and were incubated overnight and throughout day 0 in batches of 15 on 1 mM D,L-selenomethionine (+——+), 1 mM O-methylhomoserine (□——□), 1 mM L-methionine (○——○), or distilled H_2O (△——△).

and selenomethionine with oxygen in place of the sulfur or the selenium, respectively. In some experiments, O-methylhomoserine enhanced ethylene synthesis slightly (Fig. 1); in others, it had no effect.

Selenoethionine, like selenomethionine, enhanced ethylene formation in flower tissue of *I. tricolor* (Fig. 2). In all experiments where the effects of the two selenoamino acids were compared directly, selenomethionine proved to be more effective than selenoethionine in increasing the rate of ethylene synthesis. Ethionine, the S-ethyl analog of methionine, reduced ethylene synthesis in rib segments, with L-ethionine being more potent as an inhibitor than D-ethionine (Fig. 2).

In contrast to selenomethionine and selenoethionine, selenocystine inhibited ethylene formation in flower tissue of *I. tricolor*. In a typical experiment, rib segments were excised on day −1, incubated overnight on distilled H_2O and transferred to 1 mM selenocystine on the morning of day 0. During the phase of linear ethylene production, selenocystine inhibited the rate of ethylene synthesis by 53%. The biochemical basis for this inhibition was not further investigated.

At this point, the question arose whether or not exogenously supplied selenomethionine and selenoethionine served as precursors of ethylene in senescing flower tissue. Since neither selenoamino acid is available in suitable radioactive form, this problem was investigated by determining the extent to which the specific radioactivity of ethylene was diluted when the respective selenoamino acid was applied to rib segments which had been preincubated on [U-^{14}C]methionine. The results in Table I show that both selenoamino acids diluted the specific radioactivity of ethylene, with selenomethionine being more effective than either selenoethionine or methionine. In fact, the dilution of the specific radioactivity by both selenoamino acids was greater than would have been expected if only that portion of the ethylene which was above the control level had been derived from selenomethionine or selenoethionine. Table I shows that selenomethionine and selenoethionine also enhanced ethylene formation when applied to rib segments on day 0 only. In this and other experiments in which 1 mM selenomethionine was given on day 0, there was a 2-fold promotion of ethylene synthsis. We also examined the effect of ethionine on the specific radioactivity of ethylene produced by rib segments which had been incubated on [U-^{14}C]methionine. As an inhibitor of ethylene synthesis, ethionine was not expected to dilute the specific radioactivity of ethylene very effectively, unless it was converted *in vivo* to methionine via de-ethylation and subsequent methylation. As shown in Table I, ethionine was only half as effective as methionine in reducing the specific radioactivity of ethylene. The reliability of our techniques to determine the specific radioactivity of trace amounts of ethylene had been carefully checked in earlier work (3) and also in the experiments reported here. While the total amount of ethylene produced by rib segments varied somewhat from experiment to experiment (Table I), the efficiency of selenomethionine, selenoethionine, methionine, and ethionine to dilute the specific radioactivity of ethylene was very similar in each experiment and followed always the same sequence.

In further experiments, we investigated whether the aminoethoxy analog of rhizobitoxine, a compound which inhibits ethylene formation from methionine (5, 8), also inhibited ethylene

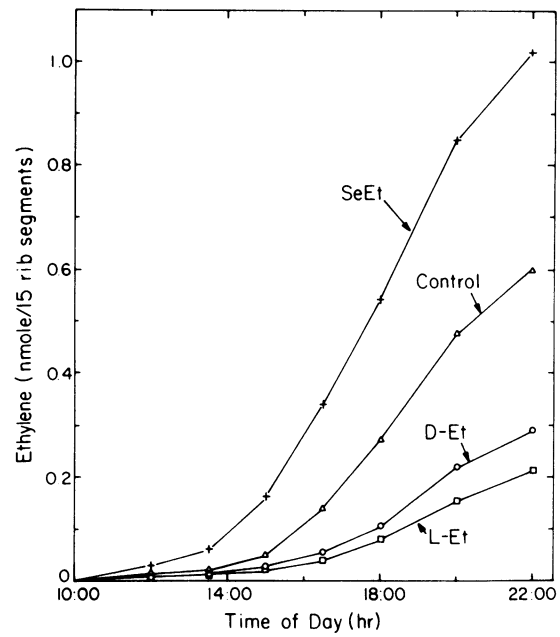

FIG. 2. Effect of ethionine and selenoethionine on ethylene synthesis in senescing rib segments of *I. tricolor*. Rib segments were excised between 4:00 and 5:00 PM on day −1 and were incubated overnight and throughout day 0 in batches of 15 on 1 mM D,L-selenoethionine (+——+), 1 mM L-ethionine (□——□), 1 mM D-ethionine (○——○), or on distilled H_2O (△——△).

Table I. Dilution of the specific radioactivity of ethylene in rib segments of I. tricolor by selenomethionine, selenoethionine, methionine and ethionine

Rib segments were excised between 4:00 and 5:00 PM on day -1 and were incubated overnight on 12 uM L-methionine-U-^{14}C (258 mCi/mmol). Between 8:00 and 9:00 AM of the following morning, the segments were rinsed to remove ^{14}C-methionine from the surface of the tissue. They were blotted, and batches of 15 segments each were incubated in 25-ml Erlenmeyer flasks on distilled water or solutions of either 5 mM D,L-selenomethionine, 5 mM D,L-selenoethionine, 5 mM L-methionine or 5 mM D,L-ethionine. Each Erlenmeyer flask contained a small vial with a filter-paper wick and saturated BaOH to trap CO_2 and was sealed with a serum-vial cap. Ethylene evolved between 6:30 and 10:30 PM was collected for the determination of its specific radioactivity.

Dilutant	Ethylene formed between 6:30 and 10:30 PM (pmol/15 segments)	Specific activity of ethylene (mCi/mmol)	Decrease of specific activity (%)	
			Found	Expected
None	157	0.96	--	--
D,L-Selenomethionine	329	0.29	70	52
D,L-Selenoethionine	228	0.49	49	31
L-Methionine	173	0.48	50	--
None	234	1.29	--	--
D,L-Ethionine	206	0.95	26	--
L-Methionine	287	0.64	50	--

synthesis in rib segments treated with selenomethionine. When selenomethionine (1 mM) and the rhizobitoxine analog (10 μM) were present continuously in the incubation medium, ethylene synthesis in the flower tissue was inhibited by 93% (Fig. 3). This value was similar to those obtained in earlier experiments where rib segments had been treated with the toxin but without selenomethionine (5). In order to determine whether treatment of rib segments with selenomethionine protected the tissue against the action of the inhibitor, rib segments were given 1 mM selenomethionine 4 hr before and continuously during treatment with a 10 μM solution of the rhizobitoxine analog. The inhibition of ethylene synthesis was 91% in segments treated with selenomethionine and the toxin as compared to 92% for rib segments which had been incubated with the toxin alone.

Ethylene production in rib segments is fairly high during the morning of day −1 and starts to subside in the early afternoon of the same day (4). Wounding of rib segments in the afternoon of day −1 induces a transient burst of ethylene synthesis (4). Rib segments excised on day −2 and incubated overnight and during the following day on 1 mM selenomethionine produced 40% less ethylene on day −1 than segments incubated on water (Table II). Similarly, treatment of rib segments with selenomethionine inhibited wound-ethylene formation by 35% (Table II).

Effect of Selenoamino Acids in Pea Stem Sections. For comparative purposes, we also investigated the effect of selenomethionine and selenoethionine on IAA-induced ethylene formation in etiolated pea stem sections. Selenoethionine tripled and selenomethionine almost doubled the amount of ethylene that was formed by auxin-treated pea stem sections (Fig. 4). In contrast to the results with rib segments, selenoethionine was always more effective than selenomethionine in enhancing ethylene production. Ethionine was not as potent an inhibitor in pea stem sections as in rib segments of morning glory flowers. Addition of 1 mM D,L-ethionine caused only a 25% inhibition of IAA-induced ethylene production (results not shown). Ethylene formation in pea stem sections was almost completely inhibited by selenocystine, however. Under experimental conditions similar to those described for Figure 4, 1 mM selenocystine reduced IAA-stimulated ethylene synthesis by 98%. In the absence of IAA, pea stem sections produced very little ethylene (Fig. 4.), even when treated with selenomethionine (results not shown).

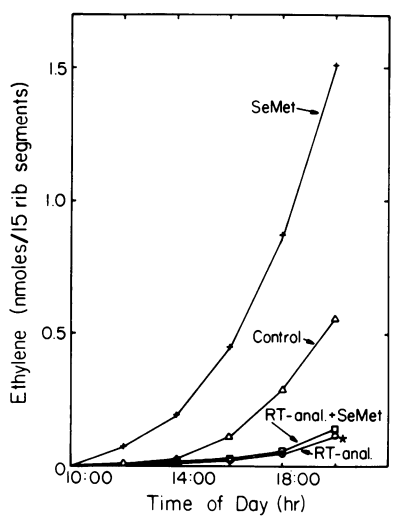

FIG. 3. Inhibition of ethylene synthesis in rib segments of *I. tricolor* by the aminoethoxy analog of rhizobitoxine. Rib segments were isolated between 4:00 and 5:00 PM on day −1 and were incubated on distilled H_2O or on 1 mM D,L-selenomethionine. At 9:00 PM of the same day, rib segments were transferred from water to either water (Δ——Δ) or to a 10 μM solution of the aminoethoxy analog of rhizobitoxine (○——○) and from D,L-selenomethionine to either 1 mM D,L-selenomethionine (+——+) or to a solution containing both 1 mM D,L-selenomethionine and 10 μM rhizobitoxine analog (□——□). Incubation on these same solutions was continued throughout day 0. A fifth batch of segments was incubated from 5:00 PM on day −1 to the end of the experiment at 8:00 PM on day 0 on a solution of 1 mM D,L-selenomethionine and 10 μM rhizobitoxine analog. The total amount of ethylene evolved under these conditions is indicated by *. Batches of 15 segments were used for each treatment.

Table II. Effect of selenomethionine on ethylene formation in intact and wounded rib segments of I. tricolor on day -1

Rib segments were excised between 4:00 and 5:00 PM on day -2 and were incubated overnight on distilled water or on 1 mM D,L-selenomethionine. At 9:00 AM of day -1, rib segments were transferred in batches of 15 to 25-ml Erlenmeyer flasks. Those segments that had been incubated overnight on water were again incubated on water; those that had been treated overnight with selenomethionine were again incubated on 1 mM D,L-selenomethionine. At 1:00 PM, one batch each of control and selenomethionine-treated rib segments was removed from its incubation flask, the segments were wounded with a file (4) and immediately returned to their original flask. The other 2 batches of control and selenomethionine-treated rib segments were left undisturbed. The flasks were sealed at 1:30 PM, and ethylene production was determined at 3:00 and 5:00 PM. Since ethylene synthesis was linear during this period, only the total amount of ethylene formed between 1:30 and 5:00 PM is given.

Treatment	Ethylene formed (pmol/15 rib segments)
Control	107
Control, wounded	415
D,L-Selenomethionine	64
D,L-Selenomethionine, wounded	268

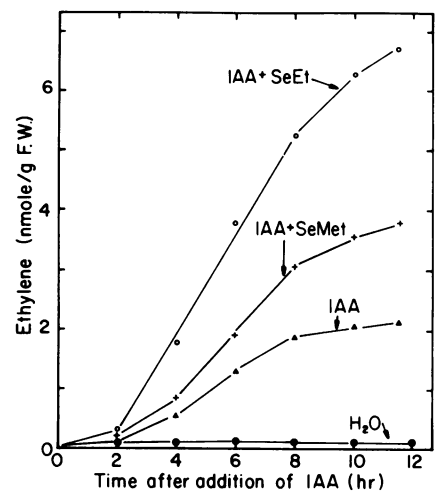

FIG. 4. Enhancement of ethylene synthesis in pea stem sections by selenoamino acids. Stem segments were isolated between 4:00 and 6:00 PM and were incubated overnight on 1 mM D,L-selenomethionine, 1 mM D,L-selenoethionine, or H_2O. At 8:00 AM of the following morning, the sections were transferred in batches of 12 to 25-ml Erlenmeyer flasks containing 1 ml of H_2O or 1 ml of 0.1 mM IAA. D,L-Selenomethionine overnight → IAA (+——+); D,L-selenoethionine overnight → IAA (○——○); H_2O overnight → IAA (Δ——Δ); H_2O overnight → H_2O (●——●).

We also confirmed with pea stem sections that the specific radioactivity of ethylene derived from [U-^{14}C]methionine was lowered when either of the two selenoamino acids was added to the incubation medium (Table III). The dilution of the specific radioactivity of ethylene by unlabeled methionine was not as high as that with selenomethionine or selenoethionine.

DISCUSSION

Our experiments dealt mainly with the effects of methionine and some of its analogs on ethylene synthesis in senescing flower tissue of *I. tricolor*. Methionine, which has been shown to be the precursor of ethylene in the morning glory system (3), enhanced

Table III. Dilution of the specific radioactivity of ethylene in pea stem sections by selenomethionine, selenoethionine and methionine

Pea stem sections were excised between 4:00 and 6:00 PM and were incubated overnight on 12 μM L-methionine-U-^{14}C (128 mCi/mmol). Between 8:00 and 9:00 AM of the following morning, the stem sections were rinsed with distilled water to remove ^{14}C-methionine from the surface of the tissue. They were blotted and batches of 12 each were floated in 25-ml Erlenmeyer flasks on 2-ml solutions of 0.1 mM IAA with or without either 1 mM D,L-selenomethionine, 1 mM D,L-selenoethionine or 1 mM L-methionine. Each Erlenmeyer flask contained a small vial with a filter-paper wick and saturated BaOH to trap CO_2 and was sealed with a serum-vial cap. Ethylene evolved between 11:00 AM and 1:00 PM was collected for the determination of its specific radioactivity.

Dilutant	Specific activity of ethylene (mCi/mmol)	Decrease of specific activity (%)
None	1.54	--
D,L-Selenomethionine	0.16	90
D,L-Selenoethionine	0.21	86
L-Methionine	0.43	72

ethylene synthesis only slightly (Fig. 1). In contrast, ethionine reduced ethylene formation by over 50% (Fig. 2). When the sulfur in methionine or ethionine was replaced by selenium, both compounds enhanced ethylene synthesis substantially (Figs. 1 and 2).

When unlabeled methionine, selenomethionine, ethionine, or selenoethionine was applied to rib segments which had been incubated previously on [U-^{14}C]methionine, the specific radioactivity of the ethylene evolved was lowered (Table I). In all experiments, selenomethionine was more effective than methionine and selenoethionine more effective than ethionine in reducing the specific activity of ethylene. The decrease in the specific radioactivity of ethylene was greater than would have been expected if only that portion of ethylene which was in excess of the control had been derived from a nonradioactive source, such as the added selenoamino acid or an endogenous precursor. As the simplest interpretation of these results, we suggest that selenomethionine and selenoethionine pass as precursors of ethylene through the methionine pool. Unless there are substantial differences in the uptake of these sulfur- and selenoamino acids, these results also indicate that selenomethionine and selenoethionine are more efficiently converted to ethylene than are methionine and ethionine, respectively. Better utilization of selenoamino acids as substrates for ethylene synthesis may thus be at least a partial explanation for the enhancement of ethylene production by these compounds. At this point, other explanations, although possible, seem less likely. For example, increased ethylene synthesis cannot have been caused by a mere expansion of the ethylene precursor pool since added methionine had only a minor stimulatory effect on ethylene production. Another possibility, namely promotion of stress-ethylene formation as a result of selenium toxicity, can probably be ruled out as well. Production of stress or wound ethylene in rib segments can only be induced on day −1, not on day 0. On day −1, selenomethionine and selenoethionine inhibited rather than enhanced ethylene synthesis in intact and in wounded rib segments (Table II). Furthermore, not all selenoamino acids stimulated ethylene synthesis, as evident from experiments where ethylene formation was inhibited by selenocystine.

Rhizobitoxine and a number of its analogs inhibit ethylene synthesis, apparently by interfering with the utilization of methionine as precursor of the gas (8). In selenomethionine-treated rib segments, ethylene formation was fully inhibited by the aminoethoxy analog of rhizobitoxine (Fig. 3). This result further supports the conclusion that selenomethionine-treated flower tissue produces ethylene via the methionine pathway.

Lieberman *et al.* (8) provided evidence that the inhibition of ethylene synthesis by rhizobitoxine and its analogs is irreversible. They also suggested that rhizobitoxine attaches itself irreversibly to the same site of the ethylene-synthesizing enzyme that otherwise binds methionine. This hypothesis was based on results of experiments with apple slices where the inhibitory action of rhizobitoxine was fully prevented by incubation of the tissue in methionine prior to and during treatment with the toxin (8). A similarly designed experiment using flower tissue of *I. tricolor* gave different results. Neither selenomethionine (Fig. 3), which may have a higher affinity to the ethylene-synthesizing enzyme than methionine, nor methionine itself (unpublished data) protected the tissue against the inhibitory action of the aminoethoxy analog of rhizobitoxine. This was true even under conditions where inhibition of ethylene synthesis by the toxin was less than 50%. The question of whether or not the precursor of ethylene and the inhibitor interact at the same site with an ethylene-generating enzyme is, therefore, still open.

In order to determine whether the enhancement of ethylene synthesis by selenomethionine and selenoethionine is restricted to senescing flower tissue or whether it is a more general phenomenon, we investigated the effect of these two selenoamino acids on IAA-induced ethylene formation in etiolated pea stem sections. The basic observations that were made with rib segments of morning glory flowers were confirmed with peas. Ethylene synthesis was greatly enhanced by both selenoamino acids, whereby selenoethionine always proved to have a higher promotive activity than selenomethionine (Fig. 4). Both selenoamino acids diluted the specific radioactivity of ethylene that was derived from [^{14}C]methionine more effectively than did unlabeled methionine (Table III). As in flower tissue of *I. tricolor*, selenocystine inhibited ethylene synthesis.

The use of selenoamino acids may prove to be very advantageous in attempts to elucidate the pathway of ethylene biosynthesis. While it is known for many plant tissues that ethylene is derived from carbons 3 and 4 of methionine, it is not clear by what mechanism methionine is converted to ethylene. Does this conversion involve the action of free radicals? Is methionine the immediate precursor or does it first have to be derivatized to another compound, such as S-adenosylmethionine (1)? Substitution of selenomethionine for methionine as precursor of ethylene may help to differentiate between these possibilities. We already know that selenomethionine is a very poor precursor of ethylene in model systems involving free radicals (Konze and Kende, unpublished data). On the other hand, selenomethionine is a much better substrate of yeast methionine adenosyltransferase than methionine (10), and seleno adenosylselenomethionine is a much better substrate for phospholipid choline synthesis in rat liver than S-adenosylmethionine (11). We intend to follow such leads in the hope of characterizing reactions in the pathway of ethylene biosynthesis.

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