Interactions of Methionine and Selenomethionine with Methionine Adenosyltransferase and Ethylene-generating Systems

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ABSTRACT

Since selenomethionine appears to be a better precursor of ethylene in senescing flower tissue of Ipomoea tricolor and in indole acetic acid-treated pea stem sections than is methionine (Konze JR, N Schilling, H Kende 1978 Plant Physiol 62: 397-401), we compared the effectiveness of selenomethionine and methionine to participate in reactions which may be connected to ethylene biosynthesis. Evidence is presented that selenomethionine is also a better substrate of methionine adenosyltransferase (ATP: methionine S-adenosyltransferase, EC 2.5.1.6) from I. tricolor, the Vmax for selenomethionine being twice as high as that for methionine. The affinity of the enzyme is higher for methionine than for selenomethionine, however. Methionine added to flower tissue together with selenomethionine inhibits the enhancement of ethylene synthesis by the seleno analog. Likewise, methionine reduces the high, selenomethionine-dependent reaction rates of methionine adenosyltransferase from I. tricolor flower tissue. On the other hand, selenomethionine is less effective as an ethylene precursor than is methionine in model systems involving oxidation by free radicals. It was concluded that activation of methionine by methionine adenosyltransferase and formation of S-adenosylmethionine are more likely to be involved in ethylene biosynthesis than is oxidation of methionine by free radicals.

In all plant tissues investigated thus far, methionine was found to be the precursor of ethylene (for reviews see refs. 13 and 22). Since methionine can be converted to ethylene in vitro via methionol (14, 20) or via the Schiff base methionine pyridoxal phosphate (10, 11) in a reaction involving free radicals, it seemed possible that during ethylene biosynthesis methionine is directly converted to ethylene by a similar radical-mediated reaction. Support for this idea came from findings that ethylene synthesis in plant tissues was inhibited by radical scavengers (3). Another possible pathway of ethylene biosynthesis involves the activation of methionine by ATP to yield SAM.5 Burg (4) was the first to propose that SAM was an intermediate in ethylene synthesis, basing this suggestion on the fact that O2 was required for the in vivo conversion of methionine to ethylene (5, 7). Experimental evidence for SAM being an intermediate in the biosynthesis of ethylene was derived from experiments using climacteric apple tissue and an inhibitor of ethylene synthesis, the aminooxy analog of rhizobitoxine. In tissue which had been treated with the rhizobitoxine analog, the level of S′-methylthiodoadenosine, a hypothetical breakdown product of SAM in the course of ethylene synthesis, was much lower than in untreated tissue (1). Whether the appearance of S′-methylthiodoadenosine is actually related to ethylene biosynthesis and what the other products are of the S′-methylthiodoadenosine-forming reaction are currently under investigation (Adams and Yang, personal communication). Since we have found selenomethionine to be a much better precursor in ethylene biosynthesis than the natural substrate methionine (12), we tried to differentiate between the two possibilities of methionine activation—radical oxidation or SAM formation—by comparing the effectiveness of selenomethionine and methionine as precursors in model systems involving free radicals and as substrates of methionine adenosyltransferase from flower tissue of Ipomoea tricolor. It has already been shown that methionine adenosyltransferase from yeast utilizes selenomethionine more effectively as substrate than methionine (15). Similar conclusions have been inferred from work on SAM-related metabolic processes in rat liver tissue (17).

MATERIALS AND METHODS

Chemicals. D,L-Methionine, D-methionine, methional, D,L-selenomethionine, D,L-ethionine, D,L-selenoethionine, S-adenosylmethionine, FMN sodium salt, ATP-Na2, pyridoxal-5-P, and DTT were purchased from Sigma Chemical Co., L-methionine from Nutritional Biochemical Corp., and ninyhdrin from General Biochemicals. The cation exchange resin AG 50W-X8 (100–200 mesh) was purchased from Bio-Rad Laboratories in the ammonium form and was converted to the Li+ form by washing with an excess of 4 M LiCl and distilled H2O. Methional and selenomethional were prepared from D.L-methionine and D.L-selenomethionine, respectively, by a Streecker degradation with ninhydrin (19, 21). The concentrations of methionol and selenomethional were determined with the iodoplatinate reagent (2), [2,8–H]ATP-Na2 was purchased from New England Nuclear. [35S]Selenomethionine from Amersham Corp.

Plant Material. Morning glory plants (I. tricolor Cav. cv. Heavenly Blue) were grown as described before (8). Rib segments were prepared from flower buds according to Kende and Hanson (9). 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.

Ethylene Determination. Ethylene formation was determined by GC as described earlier (9).

Enzyme and Chloroplast Preparations. Methionine adenosyltransferase (ATP:L-methionine adenosyltransferase, EC 2.5.1.6) was tested in homogenates of flower buds of I. tricolor on day −1. For the preparation of the homogenate, flower buds were blended in a Sorvall Omni-Mixer in 200 mM Tris-HCl (pH 7.8)
containing 0.1% 2-mercaptoethanol (v/v), and further homogenized in a glass homogenizer with a Teflon plunger. The homogenate was passed through four layers of cheesecloth and centrifuged for 12 min at 12,000g. The supernatant was used for the enzyme test. Fresh homogenates were prepared for every experiment.

Ferredoxin was prepared from spinach leaves by acetone fractionation and column chromatography on Whatman DE52 cellulose (18). Chloroplasts were isolated from spinach leaves (16) and broken by an osmotic shock.

Enzyme Assay. The methionine adenosyltransferase assay was based on that of Chiang and Cantoni (6). The standard assay contained in a total volume of 500 µl: 200 mM Tris-HCl (pH 7.8), 200 mM KCl, 10 mM MgCl₂, 1 mM DTT, 0.05% 2-mercaptoethanol (v/v), 5 mM [2,8-³²H]ATP (0.4 µCi/µmol), D,L-methionine or D,L-selenomethionine at various concentrations as indicated in the figures, homogenate from I. tricolor flower buds containing 0.7 to 0.95 mg protein. The reaction was linear for 100 min at 28°C and at the protein concentration used. After incubation for 60 min, 400 µl of the assay mixture was passed through an AG 50W-X8 (Li⁺) column (5 × 20 mm). The column was washed with 4 ml of distilled H₂O, and the resin was combusted in a Packard Tri-Carb sample oxidizer. When standards of [³²H]SAM were adsorbed to the same column and combusted with the resin, 88% of the radioactivity was recovered. This corresponded to the usual recovery of radioactivity following combustion of tritiated compounds in the sample oxidizer. Enzyme assays with homogenate and labeled ATP but no methionine or selenomethionine were conducted as blanks in every test. In separate experiments using nonlabeled ATP and [³⁸Se]selenomethionine, qualitative analysis of the reaction products by paper electrophoresis at pH 4.5 showed only one radioactive band migrating toward the cathode. This band co-migrated with SAM indicating that the seleno analog of SAM was the major reaction product carrying a net positive charge.

Model Systems for Ethylene Synthesis. Pyridoxal phosphate-mediated formation of ethylene from methionine and selenomethionine was tested according to Konze and Elstner (11). In a total of 1.0 ml of the reaction mixture contained 100 mM K-phosphate (pH 7.0), 2 mM MgCl₂, 2.5 mM NaH₂O₄, 0.32 mM pyridoxal-5-P, 0.02 mM ferredoxin, isolated chloroplast lamellae containing 5 µg Chl, 13 mM D,L-methionine or 13 mM D,L-selenomethionine. The reaction was carried out at room temperature (22°C) in 10-ml Erlenmeyer flasks which were illuminated from the bottom with red light (2 × 10⁶ erg cm⁻² s⁻¹) using a Cinemoid filter (λ > 600 nm) from Klieg Bros., Long Island City, N.Y. The flasks were sealed with serum vial caps.

FMN-mediated ethylene formation from methionine and selenomethionine was determined according to Yang (22). Twenty-five-ml Erlenmeyer flasks were filled with N₂ and closed with serum vial caps. One ml of a solution containing 50 mM K-phosphate (pH 8.4), 1 mM FMN, and 1 mM D,L-methionine or D,L-selenomethionine was injected into the flasks. The reaction was immediately started by placing the flasks on a light box with white fluorescent light (General Electric Lightbox model 11FV1). Cu/ascorbate-mediated ethylene production from methionine and selenomethionine was tested according to Lieberman et al. (14). The reaction was performed in 25-ml Erlenmeyer flasks containing a total of 5.0 ml 60 mM K-phosphate (pH 6.8), 10 mM sodium ascorbate, 1 mM CuCl₂, and 1 mM D,L-methionine or 1 mM D,L-selenomethionine. The flasks were closed with serum vial caps and incubated at 25°C on a water bath shaker in the dark.

RESULTS

Comparison of Effectiveness of Methionine and Selenomethionine as Precursors of Ethylene in Model Systems. The direct conversion of methionine and selenomethionine to ethylene in the presence of pyridoxal-5-P is compared (Fig. 1). In this system, peroxide and oxygen radicals are formed by photosynthetic electron transport from water to oxygen via ferredoxin as autoxidizable electron acceptor (11). In this model system of ethylene formation, methionine was a better precursor of ethylene than selenomethionine. Figure 2 compares the effectiveness of methionine and selenomethionine as precursors of ethylene in the FMN/light system (FMN + hν → FMN⁺) (20) and Cu/ascorbate system (Cu + ascorbate + O₂ → oxygen radicals, peroxide) (14). Again, methionine was a much better precursor of ethylene than selenomethionine. Similar results were obtained with ethionine and selenoethionine when these compounds were compared as precursors of ethylene in the FMN/light system (data not shown). In vivo, selenomethionine enhanced ethylene formation in flower tissue of I. tricolor whereas ethionine inhibited ethylene production (12).

In the Cu/ascorbate and in the FMN/light systems, methionine is first deaminated and decarboxylated in a radical-mediated reaction to yield methional, which serves as the actual precursor of ethylene (14, 20). To make sure that the low rate of conversion of selenomethionine to ethylene was not limited by inefficient deamination and decarboxylation, selenomethionial was synthesized, and its ability to serve as a precursor of ethylene was compared to that of methional in the FMN/light system (Fig. 3). Again the sulfur compound was found to be the better ethylene precursor.

Comparison of the Effectiveness of Methionine and Selenomethionine as Substrates of Methionine Adenosyltransferase. If selenomethionine and methionine are converted to ethylene via SeAM and SAM, respectively, methionine adenosyltransferase

![Fig. 1. Ethylene formation from methionine pyridoxal phosphate and selenomethionine pyridoxal phosphate in a reaction with reduced ferredoxin and oxygen. Amount of ethylene formed with D,L-selenomethionine as precursor (Δ—Δ); amount of ethylene formed with D,L-methionine as precursor (Δ—Δ).](image-url)

![Fig. 2. Ethylene formation from methionine and selenomethionine in the FMN system (A) and in the Cu/ascorbate system (B). Amount of ethylene formed with D,L-selenomethionine as precursor (Δ—Δ); amount of ethylene formed with D,L-methionine as precursor (Δ—Δ).](image-url)
from *I. tricolor* flower tissue must accept selenomethionine as substrate at least as well as it accepts methionine. This expectation is based on the earlier observation that selenomethionine is more efficiently converted to ethylene by flower tissue of *I. tricolor* than is methionine (12). Figure 4 shows the rates of SAM and SeAM formation in a homogenate of *I. tricolor* flower tissue in the presence of different concentrations of methionine and selenomethionine, respectively. The rate of conversion of the artificial substrate selenomethionine was higher than that of the natural substrate methionine. This is true not only for the substrate concentrations shown in Figure 4 but also for methionine and selenomethionine concentrations as low as 25 μM (data not shown). The reaction rates with selenomethionine as substrate of methionine adenosyltransferase from *I. tricolor* were also higher than those with ethionine as substrate (data not shown). Although the maximal reaction rates for different homogenates varied, the ratios of the *V*ₘₐₓ for selenomethionine over the *V*ₘₐₓ for methionine as calculated from Lineweaver-Burk plots were always about 2 (Fig. 4) or even higher (Fig. 5). The apparent *Kₘ* values for D,L-methionine and D,L-selenomethionine calculated from experiments with four different homogenates were 0.3 to 0.5 mM and 0.6 to 0.8 mM, respectively. This means that the rate of conversion of selenomethionine to SeAM is higher than the conversion of methionine to SAM, even though the affinity of methionine adenosyltransferase is higher for methionine than for selenomethionine. As a consequence of these properties of methionine adenosyltransferase, it is expected that methionine added to a reaction mixture containing high levels of selenomethionine will slow the rate of product formation to a rate approximating the *V*ₘₐₓ for methionine. Under these conditions, methionine would act as competitor of selenomethionine. It would be preferentially bound to the enzyme, while its conversion to the reaction product would be relatively slower. Results of experiments testing this hypothesis are given in Figure 5. This figure shows the *V*ₘₐₓ for methionine and selenomethionine (---) and the effect of different methionine concentrations on the reaction rate at the constant level of 4 mM selenomethionine (-----). As predicted, addition of D,L-methionine decreased the rate of product formation. The extent of this decrease depended on the concentration of methionine. High methionine concentrations reduced the rate almost to the *V*ₘₐₓ for methionine. L-Methionine was most effective in reducing the selenomethionine-dependent reaction rate followed by D,L-methionine and D-methionine, which had only a small effect (data not shown).

Reduction of Selenomethionine-enhanced Ethylene Production by Methionine. In order to see whether the stimulatory effect of selenomethionine on the activity of methionine adenosyltransferase and on ethylene synthesis exhibits similar characteristics, we examined the effect of methionine on selenomethionine-enhanced ethylene formation in senescing flower tissue of *I. tricolor*. The high levels of ethylene formed in the presence of selenomethionine were reduced to the level of ethylene produced by methionine-treated tissue when methionine (0.2–1.0 mM) was added to the incubation medium containing 0.5 mM selenomethionine (Fig. 6). This inhibition of ethylene synthesis was specific for methionine as concentrations of selenomethionine above 0.5 mM further enhanced ethylene synthesis. Thus, methionine overrides the enhancement of ethylene synthesis by selenomethionine, just as it overrides the selenomethionine-dependent activity of methionine adenosyltransferase.

DISCUSSION

The assumption that during ethylene biosynthesis methionine or its Schiff base is directly converted to ethylene by oxygen radicals is mainly based on the ability of methionine to serve as a precursor of ethylene in *in vitro* radical-generating systems. Figures 1 to 3 show that selenomethionine, a methionine analog with selenium in the place of sulfur, is a poor precursor of ethylene in such model systems when compared to methionine. These results fail to explain why selenomethionine is more efficient as precursor of ethylene than methionine in *I. tricolor* flower tissue and IAA-
Fig. 6. Reduction of selenomethionine-enhanced ethylene production by methionine in rib segments of I. tricolor flowers during senescence. Rib segments were excised between 4:00 and 5:00 PM on day -1 and were incubated overnight and throughout day 0 in 25 ml Erlenmeyer flasks on distilled H2O (O); 0.5 mM and 1.0 mM D,L-selenomethionine (O--O); 1 mM D,L-methionine (+); 0.5 mM D,L-selenomethionine plus 0.2 to 1.0 mM D,L-methionine (D--D). Figure shows the total amount of ethylene produced by rib segments between 10:30 AM and 11:15 PM.

treated etiolated pea stem sections. They suggest that the radical model systems do not mimic ethylene biosynthesis and that ethylene formation in vivo does not occur via direct conversion of methionine to ethylene by oxygen radicals.

On the other hand, similar characteristics of both methionine adenosyltransferase from I. tricolor flowers and ethylene formation in I. tricolor flowers suggest that the second possibility, namely methionine activation by formation of SAM, may be involved in ethylene biosynthesis in I. tricolor flower tissue. Both methionine adenosyltransferase in vitro and the ethylene-forming system in vivo turn over the artificial substrate selenomethionine at least twice as fast as the natural substrate methionine (see ref. 12 and Fig. 4). However, the affinity of methionine adenosyltransferase is higher for methionine than for selenomethionine (Km selenomethionine > Km methionine) and the same appears to be true for the ethylene-generating system. This has been derived for methionine adenosyltransferase from Lineweaver-Burk plots and is consistent with the finding that the reaction rates of methionine adenosyltransferase in vitro and of the ethylene-forming system in vivo, both operating with selenomethionine as substrate, are decreased by addition of more substrate in the form of methionine but not if the additional substrate offered is selenomethionine (Figs. 5 and 6). If the ratio of the methionine concentration over the selenomethionine concentration becomes high enough, methionine adenosyltransferase and ethylene biosynthesis are not affected by selenomethionine any more. When both selenomethionine and methionine are present in free radical-generating reaction mixtures, the results are opposite to those found with methionine adenosyltransferase and the ethylene-generating system in I. tricolor. Selenomethionine reduces the amount of ethylene formed from methionine by oxygen and FMN radicals (data not shown).

On the basis of the results presented above it appears unlikely that, in vivo, methionine or its Schiff base is directly converted to ethylene by free oxygen radicals. Our data suggest that the second proposed pathway of ethylene formation with SAM as intermediate is the one which operates in senescing flower tissue of I. tricolor and probably in other tissues in which ethylene production is stimulated by selenomethionine. Those tissues include etiolated pea stem sections (12), petals of the ephemeral flower Trandescantia clone 03 (Suttle and Kende, unpublished results), and climacteric apple tissue (Konze and Kende, unpublished results).

LITERATURE CITED
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