# CONVERSION OF METHIONINE TO ETHYLENE IN VEGETATIVE TISSUE AND FRUITS*

Stanley P. Burg and C. O. Clagett

Program in Cellular and Molecular Biology
University of Miami School of Medicine

and

Department of Biochemistry
Pennsylvania State University

Received February 17, 1967

Methionine is converted to ethylene both in apples (Lieberman et al., 1966) and model systems (Lieberman et al., 1965; Yang et al., 1966). We find this to be the major pathway of methionine metabolism in vegetative tissue, and also in apple and banana fruit sections. The $C_1$ of methionine is released as $CO_2$, $C_3$-$C_4$ as ethylene, and the methyl carbon, sulfur and $C_2$ retained in the tissue and widely metabolized.

## EXPERIMENTAL

### Experiments with Pea Stem Sections

Subapical 10 mm stem sections cut from 7 day old etiolated pea seedlings were incubated in 2% sucrose (w/v), $5 \times 10^{-6}$ M $CoCl_2$, $5 \times 10^{-2}$ M potassium phosphate buffer (pH 6.8), $10^{-3}$ M indole-3-acetate (IAA), and various concentrations of L-methionine or L-ethionine. Flasks were sealed with vaccine caps and shaken in the dark for 18 hours at 24° before ethylene was measured by gas chromatography (Burg and Burg, 1964). Conversion of $^{14}$C-methionine to ethylene

* This investigation was supported by research grants EF - 00214 and EF - 00782 from the U.S.P.H.S., Division of Environmental Engineering and Food Protection, and was carried out while S. P. Burg was recipient of Career Research Development Award 1-K3-GM-6871 from the U.S.P.H.S.

and $CO_2$ was studied as described in Table 1. Total volatile counts released from $^{35}S$-methionine was determined by the method used for $CO_2$ except samples were not passed through a dry ice trap.

Experiments with Fruits

McIntosh apples: Apples were sampled and treated by the method of Burg (1959) in which the tissue was soaked in the isotope for 1 hour, rinsed in 2% KCl, blooted dry and sealed in flasks. Under these conditions the rate of ethylene production was unimpaired, whereas it was inhibited by 90% when the tissue was left in the soaking solution as in the experiments of Lieberman et al., (1966). Total volatile counts lost during the soak period represented an insignificant value relative to that in ethylene and $CO_2$ during 1 hour after the tissue was dried. Ethylene and $CO_2$ were analyzed after 1 hour to minimize isotope redistribution, and the tissue was killed by grinding in 80% methanol. The alcohol soluble fraction was passed through IR-120 ($H^+$) and IRA-400 ($OH^-$) columns. Both columns were eluted with 4 column volumes of 4 N HCl and the eluates were dried in vacuo at 40°C. The fractions were subjected to 1 and 2-dimensional chromatography in various solvents. Radioactivity in short segments of 1-dimensional strips was measured both by scintillation and gas flow counting and 2-dimensional papers were radioautographed. Uptake of methionine was calculated from the sum of the counts recovered in ethylene, $CO_2$, alcohol soluble and alcohol insoluble fractions; this total minus the methionine and methionine sulfoxide recovered in 2-dimensional chromatograms gave a value for the methionine metabolized.

Bananas: Several 2 mm thick x 1 cm diameter slices cut from green Gros Michel bananas were weighed and sealed in a flask containing 2% sucrose (w/v), 0.1 M phosphate buffer (pH 6.8) and either 10 µMoles of an L amino acid(or non isomeric compound) or 20 µMoles of a DL-racemate. Ethylene was measured after 18 hours in the dark at 24°C.

## RESULTS

Conversion of Methionine to Ethylene

Vegetative tissue: Pea stem sections pretreated with $10^{-6}$ M IAA evolve

TABLE I

Auxin Stimulated Conversion of L-Methionine-U-$^{14}$C to Ethylene in Etiolated Pea Stem Segments

| Pretreatment (IAA molarity) | L-methionine-U-$^{14}$C Molarity | S.A. (mC/mM) | Ethylene dpm | Ethylene μL | Carbon dioxide dpm | Carbon dioxide μL |
|---|---|---|---|---|---|---|
| $10^{-6}$ | $2.4 \times 10^{-6}$ | 180 | 0 | 1.6 | 3690 | ... |
| $10^{-3}$ | $2.4 \times 10^{-6}$ | 180 | 366 | 63 | 6470 | ... |
| $10^{-3}$ | $1.0 \times 10^{-3}$ | 0.54 | 244 | 87 | 5140 | 1200 |

Fifty 10 mm pea stem segments were pretreated for 2 hours with 2% sucrose (w/v), $5 \times 10^{-6}$ $CoCl_2$ and the indicated amount of IAA in $5 \times 10^{-2}$ M potassium phosphate buffer (pH 6.8). Sections were then transferred to flasks containing 4 ml of either $10^{-3}$ M or $2.4 \times 10^{-6}$ M L-methionine-U-$^{14}$C in phosphate buffer, and after 2 hours the total production and radioactivity in $CO_2$ and ethylene determined (Burg and Burg, 1964). A flask containing $10^{-3}$ M $C^{14}$-methionine (0.54 mC/mM) without tissue evolved 1000 dpm in $C^{14}O_2$ during an hour, but no detectable or radioactive ethylene. The $C^{14}O_2$ values in the table have not been corrected for this spontaneous breakdown.

TABLE II

Conversion of $^{14}$C-Methionine to $CO_2$ and Ethylene by Apple Sections

| Carbon position in methionine | Relative contribution to Ethylene | Relative contribution to Carbon dioxide |
|---|---|---|
| carboxyl | 0.004 | 0.962 |
| 2 + 3 + 4 | 0.989 | 0.016 |
| methyl | 0.007 | 0.022 |

L-isotopes were administered at $2.8 \times 10^{-4}$ M (6.8 μC/μM). The relative contribution of the carboxyl and methyl carbons is calculated by comparing one-sixth the specific activity of the ethylene and $CO_2$ which resulted in each case with that from methionine-U-$C^{14}$. The contribution of 2 + 3 + 4 is that part of the activity in $CO_2$ and ethylene not accounted for by the carboxyl and methyl carbons of methionine. The carboxyl and methyl carbons of D-methionine were also converted to $CO_2$, but with only half the yield of the comparable carbons in the L-isomer.

---

$^{14}CO_2$ but no radioactive ethylene when they are transferred to $^{14}$C-methionine, whereas sections pretreated with $10^{-3}$ M IAA produce labeled ethylene (Table I) because auxin induces an enzyme needed in the reaction (Abeles, 1966). Added methionine increased the rate of ethylene formation maximally at a concentration of $10^{-3}$ M when the specific activity of the derived ethylene (27.3 μC/mM) was 13%

of the value theoretically possible assuming mole for mole conversion of methionine to ethylene. When the concentration of methionine was too low to enhance ethylene formation ($2.4 \times 10^{-6}$ M methionine, Table I) extensive isotope dilution occurred and the specific activity of the ethylene (60 μC/mM) was only 0.03% of that theoretically possible. Model systems also convert methionine to ethylene but the mechanism is not identical to that in pea tissue. For example, ethionine inhibits ethylene formation in pea section (also in apples, Lieberman et al., 1966) but substitutes for methionine in model systems, and stem tissue synthesizes ethylene under aerobic conditions in the dark in contrast to the requirement for anaerobiosis and light in the FMN mediated system (Yang et al., 1966). However, model systems produce methyl mercaptan and related volatile products, whereas pea stem sections pretreated with $10^{-3}$ M IAA evolved few volatile counts when incubated with 19.3 μC of L-methionine-$^{35}$S (154 mC/mM). A comparison of the $^{35}$S and $^{14}$C data indicates that less than one volatile S-atom is released per 20 molecules of ethylene formed.

Apples: In apple tissue the specific activity of ethylene derived from $10^{-3}$ M L-methionine-U-$^{14}$C was 17% of that theoretically possible assuming mole for mole conversion, and the rate of ethylene formation was enhanced by 60%. Concentrations of methionine lower than $10^{-5}$ M did not simulate ethylene formation and the specific activity of the derived ethylene was only a few tenths of a percent that of the $^{14}$C-methionine. However, under these conditions the greatest overall conversion of methionine to ethylene occurs. For example, after $2.4 \times 10^{-7}$ M methionine was administered, during a subsequent hour 80% of the methionine metabolized by the tissue was utilized for ethylene synthesis. In a dozen experiments with methionine-U-$^{14}$C the yield of radioactivity in ethylene relative to that in $CO_2$ was $1.7 \pm 0.3$, suggesting that only one $CO_2$ arises per ethylene molecule formed. Inclusion of 10% unlabeled $CO_2$ did not alter this result proving that $C^{14}O_2$ fixation is not a problem. Lieberman et al., (1966) found apples to form ethylene from $C_3$-$C_4$ of methionine and we have obtained similar results in studies utilizing DL-methionine-1-$^{14}$C (57.3 μC/14.5 μM), DL-methionine-2-$^{14}$C (62.2 μC/21.8 μM), L-methionine-methyl-$^{14}$C (66.6 μC/9.7 μM) and L-methionine-U-$^{14}$C (24.4 μC/7 μM).

These amounts of isotope were administered to duplicate 13.5 g lots of apple tissue and during 1 hour each produced 1.6 μL of ethylene containing 54, 84, 29, and 36,500 dpm respectively. Results of a similar experiment are included in Table II along with data on $C^{14}O_2$ production, which show that ethylene is formed exclusively from $C_3$-$C_4$ and $CO_2$ from $C_1$. This $CO_2$ probably does not result from decarboxylation of a 2-carbon piece ($C_1$-$C_2$) for no labeled glycine, glyoxylate or glycolate was found in the tissue, and applied glyoxylate (Table III) did not reduce the total $C^{14}O_2$ derived from methionine-U-$^{14}$C. The FMN catalyzed model system also produces ethylene from $C_3$-$C_4$ and $CO_2$ from $C_1$; $C_2$ gives rise to formate, N to ammonia and S to methyl mercaptan and dimethyl sulfide (Yang et al., 1966). However, apple tissue exposed to 39 μC of L-methionine-$^{35}$S (154 mC/mM) produced few volatile counts. The $^{35}$S was recovered mainly in cationic components such as methionine and S-adenosyl methionine, and with trace quantities in neutral and anionic compounds. The methyl carbon of methionine gave rise to some count in the pectin and lipid fractions and accompanied the S of methionine in S-adenosyl methionine and a neutral compound.

Bananas: Ethylene formation in banana slices was stimulated almost equally by L-methionine (+67%), DL-homocysteine (+61%), DL-homoserine (+59%), and methionine hydroxy analogue (+59%); DL-allocystathionine (+20%) and DL-α amino butyrate (+18%)

TABLE III

Relative Efficacy of Several Compounds in Reducing the Specific Activity of Ethylene Derived from $^{14}$C-Methionine in Bananas

| Compound | Molarity | % change in S.A. of ethylene* |
|---|---|---|
| L-methionine | $8 \times 10^{-5}$ | -88 |
| " | $7 \times 10^{-4}$ | -94 |
| " | $1 \times 10^{-3}$ | -98 |
| DL-homoserine | $1 \times 10^{-3}$ | -41 |
| L-threonine | $1 \times 10^{-3}$ | +3 |
| glycoxylic acid | $1 \times 10^{-3}$ | -3 |

* Relative to the specific activity obtained with $2.4 \times 10^{-7}$ M L-methionine-U-$^{14}$C (234 mC/mM) without any added unlabeled compound.

gave slight stimulation, whereas L-threonine and β-alanine were without effect and γ-methyl mercaptopropionaldehyde (-27%) was slightly inhibitory. Similar but less consistent results were obtained with apple tissue, indicating that β-alanine is not a precursor of ethylene as proposed by Thompson and Spencer (1966), and suggesting that methionine might be converted to homoserine via homocysteine enroute to ethylene. However, the data in Table III show unlabeled methionine to be far more effective than homoserine in reducing the specific activity of ethylene derived from $^{14}C$ methionine, and therefore methionine must be closer than homoserine to the immediate precursor of ethylene.

REFERENCES

Abeles, F., Plant Physiol., 41, 585 (1966).

Burg, S. P., Arch Biochem. Biophys., 84, 544 (1959).

Burg, S. P., and Burg, E. A., Nature, 203, 869 (1964).

Lieberman, M., Kunishi, A. T., Mapson, L. W., and Wardale, D. A., Biochem. J., 97, 449 (1965).

Lieberman, M., Kunishi, A., Mapson, L. W., and Wardale, D. A., Plant Physiol., 41, 376 (1966).

Thompson, J. E., and Spencer, M., Nature, 210, 595 (1966).

Yang, S. F., Ku, H. S., and Pratt, H. K., Biochem. and Biophys. Res. Comm., 24, 739 (1966).