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Short Communication

Precursors of Ethylene[1]

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Two pathways for biosynthesis of ethylene in higher plants have been postulated (10). One is associated with the breakdown of peroxidized linolenic acid and the other involves the degradation of methionine. Although the formation of ethylene from peroxidized linolenate has been demonstrated in model systems catalyzed by Cu^{2+}, oxygen and ascorbic acid (10) and by an apple extract in the presence of oxygen and ascorbic acid (4), there is no direct evidence that it occurs in plant tissues. The conversion of methionine to ethylene in model systems (8, 19) and in plant tissues (2, 9) has been demonstrated. In the FMN-light mediated model system (19), it has been established that methionine is converted to ethylene *via* methional (β-methylthiopropionaldehyde) as an intermediate. Enzymic conversion of methionine analogs to ethylene catalyzed by peroxidase has been elucidated recently (5, 6, 11, 12, 15, 17); α-keto-γ-methylthiobutyric acid and methional, but not methionine, are the active substrates. A chemical mechanism accounting for such enzymic formation of ethylene has been described (15–18). On the basis of this information, Yang (16) has proposed the following scheme for the biosynthesis of ethylene in plants:

methionine → α-keto-γ-methylthiobutyric acid → methional → ethylene.

In order to test the proposed pathways, radioactive linolenic acid and the appropriate radioactive methionine analogs were fed to apple tissue. None of these suggested precursors of ethylene was converted to ethylene as effectively as was methionine.

Materials and Methods

Materials. Linolenic acid-U-^{14}C, L-methionine-U-^{14}C and DL-methionine-^{3}H were obtained from Applied Science Laboratories, New England Nuclear Corporation and International Chemical & Nuclear Corporation, respectively. α-Keto-γ-methylthiobutyric acid-U-^{14}C was prepared enzymically from L-methionine-U-^{14}C, and methional-^{3}H was prepared from DL-methionine-^{3}H by the Strecker reaction with ninhydrin according to the procedures described elsewhere (17). β-Methylthiopropylamine-^{3}H was prepared from DL-methionine-^{3}H with acetophenone (14). It has been established that only the ethylene moiety (carbons 3 and 4) of methionine are converted to ethylene both in plant tissues (2, 9), and in the FMN-light model system (19). For estimation of the specific radioactivity of the ethylene moiety of methionine, DL-methionine-^{3}H (109 μc/μmole) was converted to ethylene by FMN and light (19), and the specific radioactivity (46 μc/μmole) of the ethylene thus produced was determined by gas radiochromatography as described below.

Feeding Experiments With Apple Tissues. Plugs (1.0 cm in diameter and 2.0 cm in length) were cut from a mature apple fruit with a corkborer and razor blade as described previously (1). The radioactive substrates in 2% KCl were introduced into apple tissue either by a vacuum injection technique similar to that employed by Frankel *et al.* (3), or by soaking (2). The plugs were then sealed in 25 ml Erlenmeyer flasks.

Gas Analysis. Samples of the gas phase of the flasks were withdrawn for estimation of total ethylene and of radioactive ethylene and carbon dioxide by gas-chromatograph and gas radiochromatography, as previously described (1).

Results and Discussion

The substrates of interest were tested as precursors of ethylene in apple tissue, and the efficiency in each case was compared to that of methionine (table I). The failure of conversion of linolenic acid-U-^{14}C to ethylene appears not due to inadequate uptake, since it was efficiently converted to carbon dioxide. It is therefore concluded that linolenic acid does not

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function as a physiological precursor of ethylene in this tissue. Lieberman and Kunishi (7) have suggested that propanal, which is a decomposition product of peroxidized linolenic acid and an effective precursor of ethylene in the model system, may be an intermediate in the conversion of linolenic acid to ethylene *in vivo*. Baur and Yang (1) have since shown that propanal is not a precursor of ethylene in apple tissue. In this regard it is pertinent to note that a large amount of ethane was produced along with ethylene when linolenic acid or propanal was used as substrate either by the model system consisting of Cu^{2+} and ascorbate (10), or by the apple enzyme system with linolenic acid as substrate (4). The facts that linolenic acid or propanal yields both ethylene and ethane while methionine yields only ethylene (14, 16) have been rationalized in chemical terms (1, 18). In view of the fact that intact fruits produce very little ethane (the ratio of ethylene to ethane production in whole apple is about 14,000:1, ref. 13), it is most unlikely that ethylene is derived from linolenic acid.

For the conversion of methionine to methional, 2 biochemical routes are possible. One is by oxidative deamination or transamination (*via* α-keto-γ-methylthiobutyric acid) followed by decarboxylation. The other involves the decarboxylation (*via* β-methylthiopropylamine) followed by oxidation of the corresponding amine to aldehyde. Although α-keto-γ-methylthiobutyric acid was an efficient precursor, it was not as efficient as methionine. β-Methylthiopropylamine was inactive and, surprisingly, methional was a poor precursor. It is possible that methional, when supplied to apple tissue, may be converted into an inactive form. It is also possible that the methional which is active in ethylene biosynthesis is an enzyme-bound form, and that exogenously supplied methional is not converted into the active intermediate. However, the present data do not support the hypothesis that methionine is converted to ethylene through methional. Since methionine and α-keto-γ-methylthiobutyric acid are interconvertible through transamination, it is not surprising that both substrates, when incubated for 2 hr, converted to ethylene with nearly equal efficiency as shown in table I. When the incubation period was reduced to 30 min, however, methionine was found to be converted to ethylene nearly twice as efficiently as α-keto-γ-methylthiobutyrate. These data suggest that methionine is a more direct precursor of ethylene in this system than is α-keto-γ-methylthiobutyrate.

As in the model system, methionine sulfoxide was an inefficient precursor (19). The inefficiency of this conversion may be due to a limited reduction of methionine sulfoxide to methionine as a prerequisite to conversion to ethylene. Homoserine, which is a

Table I. *The Conversion of Labeled Substrates to Ethylene by Apple Slices*

In Expt. 1, 5 or 6, the substrate was dissolved in 0.1 ml of 2% KCl solution and then injected under vacuum into 1 plug of apple slice. In Expt. 2, 3 or 4, 2, 1 or 2 plugs of apple slice were soaked for 1 hr, respectively, in 2.8 ml, 0.6 ml or 1.6 ml of 2% KCl solution containing the radioactive substrate. An apple was used for each experiment. Linolenic acid was converted to ammonium salt before dissolving in 2% KCl. Percent of conversion of the substrate to ethylene was calculated assuming that only L-isomer and the ethylene moiety of the substrate molecule are converted to ethylene.

Expt.	Substrate		Incubation time		Ethylene			CO_2
		μc	*μc/μmole*	*hr*	*mμmole*	*mμc*	*% conversion*	*mμc*
1	Linolenic acid-U-^{14}C	1.3	630	2	5.2	0	0	10
	DL-Methionine-^{3}H	1.25	107	2	5.7	9.5	3.5	0
2	α-Keto-γ-methylthiobutyric acid-U-^{14}C	1.4	50	2	8.8	17	3.0	14
	L-Methionine-U-^{14}C	1.4	50	2	9.5	21	3.8	14
3	Methional-^{3}H	23	107	1	2.0	2.0	0.02	0
	DL-Methionine-^{3}H	23	107	1	2.9	21	0.42	0
4	β-Methylthiopropylamine-^{3}H	13	107	3	11.0	0	0	0
	DL-Methionine-^{3}H	17	107	3	12.1	46	1.3	0
5	L-Methionine sulfoxide-U-^{14}C	0.96	220	3	6.3	3.5	0.91	...
	L-Methionine-U-^{14}C	0.82	220	3	6.9	21	6.4	...
6	DL-Homoserine-4-^{14}C	2.3	10	4.5	20	36	3.1	6.3
	L-Methionine-U-^{14}C	2.2	11	4.5	15	96	11	50

Table II. *Reduction of ^{3}H-ethylene Production From* DL-*methionine-^{3}H in Apple Slices by* D- *or* L-*methionine*

Plugs (1 × 2 cm) of apple tissue were fed with a syringe under vacuum 0.1 ml of 2% KCl solution containing 7.7 μc of DL-methionine (107 μc/μmole) of D- or L-methionine as indicated. Incubation time was 3 hr.

Substrate	Ethylene	
	mμmole	*dpm* × *10* ^{3}
DL-Methionine-^{3}H	9.8	93
DL-Methionine-^{3}H + L-Methionine	12.2	7.2
DL-Methionine-^{3}H + D-Methionine	10.3	54
None	7.6	...

close precursor of methionine, was also found to be converted to ethylene. Since the conversion of homoserine to ethylene is less efficient than that of methionine, it is concluded that homoserine is not a closer precursor of ethylene than is methionine.

The data in table II show that unlabeled L-methionine is far more effective than D-methionine in reducing the production of radioactive ethylene from DL-methionine-^{3}H. These results indicate that the conversion of methionine to ethylene is stereospecific for the L-isomer. The apparent activity of D-methionine is congruent with stereospecificity if it is assumed that a racemase catalyzes a limited conversion of D-methionine to the L-isomer.

Although the conversion of methionine to ethylene in plant tissue has been established, details of the pathway and the chemical reactions involved in the conversion remain to be elucidated.

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