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METHIONINE METABOLISM IN APPLE TISSUE IN RELATION TO ETHYLENE BIOSYNTHESIS

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Key Word Index—*Malus pumila*; Rosaceae; methionine metabolism; *S*-methylcysteine; ethylene biosynthesis.

Abstract—A comparison of the rate of ethylene production by apple fruit to the methionine content of the tissue suggests that the sulfur of methionine has to be recycled during its continuous synthesis of ethylene. The metabolism of the sulfur of methionine in apple tissue in relation to ethylene biosynthesis was investigated. The results showed that in the conversion of methionine to ethylene the CH_3S-group of methionine is first incorporated as a unit into *S*-methylcysteine. By demethylation, *S*-methylcysteine is metabolized to cysteine. Cysteine then donates its sulfur to form methionine, presumably through cystathionine and homocysteine. This view is consistent with the observation that cysteine, homoserine and homocysteine were all converted to methionine, in an order of efficiency from least to greatest. For the conversion to ethylene, methionine was the most efficient precursor, followed by homocysteine and homoserine. Based on these results, a methionine-sulfur cycle in relation to ethylene biosynthesis is presented.

INTRODUCTION

ETHYLENE is a natural plant hormone initiating fruit ripening and regulating many aspects of plant growth and development.[1] In apple tissue, it is established that methionine is the major, if not the sole, precursor of ethylene,[2–6] and this conversion of methionine to ethylene represents the major pathway of methionine metabolism.[6] In an FMN-mediated photochemical reaction, methionine was shown to be efficiently degraded into ethylene and other products as represented by the following equation[7]

$$\overset{5}{CH_3}S\text{–}\overset{4}{CH_2}\text{–}\overset{3}{CH_2}\text{–}\overset{2}{CH}(NH_2)\text{–}\overset{1}{COOH} \rightarrow \tfrac{1}{2}(S\text{–}\overset{5}{CH_3})_2 + \overset{4}{CH_2}=\overset{3}{CH_2} + \overset{2}{HCOOH} + NH_3 + \overset{1}{CO_2}$$

(also $HS\text{–}CH_3$).

When methionine was fed into apple tissues, C-1 of methionine gave rise to CO_2,[2,6] C-2 to formic acid[8] and C-3 and C-4 to ethylene,[2,6] but the sulfur was not volatized and retained in the apple tissue.[6]

We have found that the methionine concentration in apple fruit was extremely low (60 μmol/kg) when compared to the ethylene production rate (5 μmol/kg-hr).[4] Apples are

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[1] H. K. PRATT and J. D. GOESCHL, *Ann. Rev. Plant Physiol.* **20,** 541 (1969).
[2] M. LIEBERMAN, A. KUNISHI, L. W. WARDALE and L. W. MAPSON, *Plant Physiol.* **41,** 376 (1965).
[3] A. H. BAUR, S. F. YANG, H. K. PRATT and J. B. BIALE, *Plant Physiol.* **47,** 696 (1971).
[4] S. F. YANG and A. H. BAUR, *Proc.* 1971 *Plant Growth Regulators Congress*, Canberra, Australia, in press.
[5] L. D. OWENS, M. LIEBERMAN and A. KUNISHI, *Plant Physiol.* **48,** 1 (1971).
[6] S. P. BURG and C. O. CLAGETT, *Biochem. Biophys. Commun.* **27,** 125 (1967).
[7] S. F. YANG and H. S. KU and H. K. PRATT, *J. Biol. Chem.* **242,** 5274 (1967).
[8] K. J. SIEBERT and C. O. CLAGETT, *Plant Physiol.* **44,** S-30 (1969).

known to sustain a high rate of ethylene production for months. If the sulfur were lost to the atmosphere during the ethylene production, then there would be a shortage of sulfur. These data indicate that the sulfur atom of methionine must be conserved and recycled during the continuous synthesis of ethylene. In this paper, we wish to present evidence showing the recycling of methionine sulfur in the apple tissue during its ethylene biosynthesis.

RESULTS AND DISCUSSION

There are two possibilities which may explain the failure to detect sulfur as methanethiol or dimethyldisulfide in the biosynthesis of ethylene. One explanation is that neither methanethiol nor dimethyldisulfide is formed during the degradation of methionine to ethylene; the other explanation is that methanethiol or dimethyldisulfide are formed but these compounds are efficiently converted to nonvolatile forms. The latter possibility was tested experimentally. When methanethiol-^{14}C or -^{35}S was introduced into sealed flasks in which apple plugs were incubated, more than 95% of the nonvolatile radioactivity was recovered as *S*-methylcysteine (Table 1). *S*-Methylcysteine was identified by paper cochromatography, paper coelectrophoresis and oxidation to *S*-methylcysteine sulfoxide with H_2O_2 (see Experimental). Although methionine was also a product, its formation was extremely low as compared to that of *S*-methylcysteine. When apple plugs were injected with 1 μmol of L-homoserine or L-methionine before the incubation with methanethiol-^{35}S, the incorporation of the label into methionine was not affected. The lower yield of *S*-methylcysteine from $CH_3{}^{35}SH$ than that from $^{14}CH_3SH$ (Table 1) was probably due to the lower specific radioactivity of $CH_3{}^{35}SH$ employed. Since both ^{14}C and ^{35}S of CH_3SH were converted to *S*-methylcysteine at comparable rates, it is concluded that they were incorporated as a unit into *S*-methylcysteine.

TABLE 1. METABOLISM OF METHANETHIOL-^{35}S AND -^{14}C IN APPLE TISSUE*

Substrate	Non volatile radioactivity			
	Aqueous phase μCi	*S*-Methylcysteine μCi	Methionine μCi	CO_2 μCi
$^{14}CH_3$–SH (10 μCi, 0·9 μmol)	1·93	1·7	0·001	0·77
CH_3–^{35}SH (8 μCi, 2·0 μmol)	0·55	0·45	0·002	—

* Two plugs each of apple tissue (2·6 g) were incubated in sealed 50 ml Erlenmeyer flasks. Methanethiol was injected into the gas phase. After incubation for 8 hr, the tissues were extracted with $CHCl_3$–MeOH–H_2O.

Giovanelli and Mudd[9] and Granroth[10] have demonstrated the enzymic formation of *S*-methylcysteine and methionine through direct methylthiolysis of *O*-acetylserine and *O*-acetylhomoserine with methanethiol by Reactions (1) and (2):

$$O\text{-Acetylserine} + CH_3SH \rightarrow S\text{-methylcysteine} + \text{acetate} \quad (1)$$

$$O\text{-Acetylhomoserine} + CH_3SH \rightarrow \text{methionine} + \text{acetate} \quad (2)$$

[9] J. GIOVANELLI and S. H. MUDD, *Biochem. Biophys. Res. Commun.* **31**, 275 (1968).
[10] B. GRANROTH, *Annales Academiae Scientiavum Fennicae* Series A II Chemica (1970).

It should be noted that in the above sulfuration reactions catalyzed by the spinach enzymes, *S*-methylcysteine was formed at a much higher rate than was methionine.[9] This agrees with the present *in vivo* data shown in Table 1.

If, during the biosynthesis of ethylene, a CH_3S-fragment is released from methionine and later incorporated into *S*-methylcysteine, then one may anticipate *S*-methylcysteine as a metabolite of methionine. Table 2 shows that this is the case. When L-methionine-methyl-^{14}C or -^{35}S were fed to apple tissue which was incubated for 5 hr, radioactive *S*-methylcysteine was found in the extracts. It is important to note that the methyl group of methionine was incorporated as efficiently as was the sulfur atom. Apparently, during the conversion of methionine to *S*-methylcysteine, the CH_3S-group was transferred as a unit. Similar results have been reported with garlic plants by Sugii *et al.*[11] who observed an efficient recovery of ^{35}S in *S*-methylcysteine after feeding methionine-^{35}S. They concluded that CH_3S-group of *S*-methylcysteine arose from methionine degradation as in animals and microorganisms.[12] It is interesting to note that when $^{14}CH_3SH$ or methionine-methyl-^{14}C was administered to apple tissues, both substrates were converted to $^{14}CO_2$. The biochemical pathway of CO_2 formation from these substrates is unknown. In view that $^{14}CH_3SH$ was converted to CO_2 more efficiently than was methionine-methyl-^{14}C, it is suggested that the methyl group of methionine is converted to CO_2 via CH_3SH or its derivatives as an intermediate.

TABLE 2. METABLLISM OF L-METHIONINE-METHYL-^{14}C AND -^{35}S IN APPLE TISSUE*

Substrate	Metabolites			
	S-Methylcysteine mμCi	Cysteine plus cystine mμCi	Methionine μCi	CO_2 mμCi
L-Methionine-methyl-^{14}C	18	0·0	0·95	16
L-Methionine-^{35}S	22	3·1	0·91	—

* Two plugs of apple tissue per experiment were injected with 0·1 ml 2% KCl containing 1·3 μCi and 27 mμmol of L-methionine-methyl-^{14}C or 1·1 μCi and 52 mμmol of L-methionine-^{35}S. The tissue plugs were incubated in 25 ml Erlenmeyer flasks for 5 hr.

The next question is whether or not *S*-methylcysteine can donate its methylmercapto group or sulfur atom for the synthesis of methionine. In order to clarify this point *S*-methyl-L-cysteine-^{35}S and -methyl-^{3}H were prepared and they were administered to apple tissues (Table 3). Although both the methyl group and the sulfur atom of *S*-methylcysteine were transfered to methionine, the rate of the incorporation for the two isotopes was unequal suggesting that the methylmercapto group of S-methylcysteine is not incorporated as a unit into methionine. In the ^{35}S and ^{3}H dual-label experiment (Table 3), the ratio of $^{35}S/^{3}H$ in S-methylcysteine administered was 0·20, while the ratio of $^{35}S/^{3}H$ found in methionine was 1·6, indicating that sulfur of *S*-methylcysteine is incorporated into methionine 8 times more efficiently than the methyl group. Another major metabolite isolated

[11] M. SUGII, S. NAGASAWA and T. SUZUKI, *Chem. Pharm. Bull. Tokyo* **11**, 135 (1963).
[12] E. S. CANELLAKIS and H. TARVER, *Arch. Biochem. Biophys.* **42**, 446 (1953).

from the tissue was cysteine. That the methylmercapto group of *S*-methylcysteine is not incorporated into methionine as a unit is further supported by the results shown in Table 4.

TABLE 3. METABOLISM OF *S*-METHYL-L-CYSTEINE (SMC) IN APPLE TISSUE*

Substrate	SMC mμCi	Metabolites: Methionine mμCi	Methionine $^{35}S/^{3}H$	Metabolites: Cysteine plus cystine mμCi
SMC-methyl-^{3}H (0·7 μCi, 7 mμmol)	143	12	—	0
SMC-^{35}S (0·5 μCi, 7·6 mμmol)	210	101	—	50
SMC-methyl-^{3}H and -^{35}S (2·27 μCi, 25 mμmol, $^{35}S/^{3}H$ = 0·20)	545	75	1·6	35

* Substrates were dissolved in 0·1 ml KCl and vacuum-injected into apple plugs. Incubation time was 3 hr at 20°.

When *S*-methylcysteine labeled with methyl-^{3}H and ^{35}S which had an $^{35}S/^{3}H$ ratio of 1·1 was incubated with apple tissues for 2·5 and 5 hr, the ratio of $^{35}S/^{3}H$ in *S*-methylcysteine recovered reduced to 0·26 and 0·07, respectively, while the ratio of $^{34}S/^{3}H$ in cystine increases to more than 100. If the methylmercapto group of *S*-methylcysteine is transferred to methionine as a unit, then one may anticipate that the $^{35}S/^{3}H$ ratio of methionine recovered should be lower than that of *S*-methylcysteine administered, since the ratio of $^{35}S/^{3}H$ in *S*-methylcysteine decreased during the incubation. However, this is contrary to the data of Table 3 which shows that the ratio of $^{35}S/^{3}H$ in methionine recovered is 8 times

TABLE 4. RECOVERY OF RADIOACTIVITY IN *S*-METHYLCYSTEINE AND CYSTEINE AFTER INCUBATING APPLE PLUGS WITH *S*-METHYLCYSTEINE-METHYL-^{3}H AND -^{35}S*

Incubation (hr)	*S*-Methylcysteine mμCi	*S*-Methylcysteine $^{35}S/^{3}H$	Cysteine plus cystine mμCi	Cysteine plus cystine $^{35}S/^{3}H$
2·5	540	0·26	54	> 100
5	361	0·07	44	> 100

* Two plugs of apple tissue per experiment were injected with 0·1 ml of 2% KCl containing 25 mμmol and 1 μCi of *S*-methylcysteine labeled with methyl-^{3}H and ^{35}S. The ratio of $^{35}S/^{3}H$ was 1·1. After incubation for 2·5 or 5 hr, the tissues were extracted with $CHCl_3$–MeOH–H_2O. Amino acids were purified by Dowex 50 (H^+ form) ion exchange resin.

higher than that of *S*-methylcysteine administered. The decrease in $^{34}S/^{3}H$ ratio of *S*-methylcysteine during the incubation may be explained on the basis that the methyl group of *S*-methylcysteine is transferred to cysteine yielding ^{3}H-labeled *S*-methylcysteine and ^{35}S-cysteine as shown by Reaction (3).

$$HS\text{–}CH_2\text{–}CH(NH_2)\text{–}COOH + C^{3}H_3{}^{35}S\text{–}CH_2\text{–}CH(NH_2)\text{–}COOH \rightleftharpoons C^{3}H_3\,S\text{–}CH_2\text{–}CH(NH_2)\text{–}COOH + H^{35}S\text{–}CH_2CH(NH_2)\text{–}COOH. \quad (3)$$

Further support for such an explanation is that when DL-cysteine-3-^{14}C or L-cysteine-^{35}S

was incubated with apple tissues, they were significantly converted to *S*-methylcysteine (Table 5). Apparently, *S*-methylcysteine is formed in apple tissue by two pathways; one involves the transfer of the methylmercapto group from methionine to an acceptor, probably *O*-acetylserine (Table 2), and the other involves methylation of cysteine. Such dual pathways for the biosynthesis of *S*-methylcysteine have been proposed for allium.[10] The nature of this enzymic reaction has not been studied yet. Based on the evidences that sulfur but not the thiomethyl group of *S*-methylcysteine is transferred to methionine (Table 3) and that *S*-methylcysteine is converted to cysteine (Tables 3 and 4), it is reasonable to assume that *S*-methylcysteine is converted to methionine via cysteine. It is pertinent that Mae *et al.*[13] have recently shown that *S*-methylsysteine and its sulfoxide are metabolized to cysteine in Chinese cabbage tissue by demethylation.

TABLE 5. METHIONINE BIOSYNTHESIS IN APPLE TISSUE*

Exp.	Substrates	Products: Methionine mμCi	Methionine % conversion	Ethylene mμCi	Ethylene % conversion	*S*-Methylcystine mμCi	*S*-Methylcystine % conversion
	S-Methyl-L-cysteine-3-^{14}C (3·4 μCi, 59 mμmol)	trace	—	—	—	—	—
	S-Methyl-L-cysteine-^{35}S (2·2 μCi, 67 mμmol)	110	5·0	—	—	—	—
1	DL-Cysteine-3-^{14}C (3·6 μCi, 62 mμmol)	trace	—	—	—	252	7·0
	L-Cysteine-^{35}S (2·2 μCi, 67 mμmol)	120	5·5	—	—	210	9·5
	L-Homoserine-4-^{14}C (0·41 μCi, 41 mμmol)	40	9·8	3·9	0·9		
	L-Homocysteine-U-^{14}C (0·32 μCi, 8 mμmol)	57	18	1·8	1·1		
2	L-Homocystine-U-^{14}C (0·32 μCi, 4 mμmol)	20	6	0·5	0·3		
	L-Methionine-U-^{14}C (0·40 μCi, 8 mμmol)	—	—	19	12		

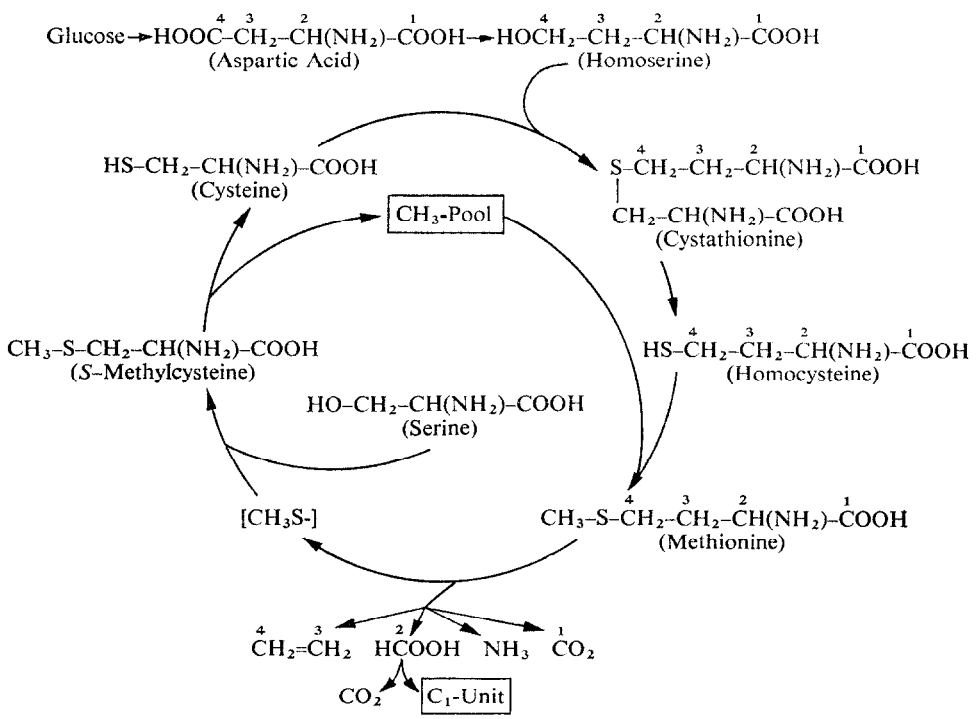
* One plug of apple tissue (1 × 2 cm, 1·4 g) was injected with 0·1 ml 2% KCl solution which contained the radioactive substrates. Incubation time was 3 hr. Products were analyzed (see Experimental); % ethylene conversion was calculated based on the assumption that only carbons 3 and 4 of methionine or its analogues are incorporated into ethylene.

The transfer of sulfur from cysteine to methionine via cystathionine and homocysteine has been implicated in plant systems[14] and the enzymes involved have been isolated from the plant sources.[15–17] If the cystathionine pathway is utilized to synthesize methionine, then one would expect that the known intermediates of this pathway are incorporated into methionine. Table 5 shows that this is the case. Both cysteine-^{35}S and *S*-methylcysteine-^{35}S were incorporated into methionine to a roughly equal extent while cysteine-3-^{14}C and *S*-methylcysteine-3-^{14}C were not. Homoserine and homocysteine are incorporated into

[13] T. MAE, K. OHIRA and A. FUJIWARA, *Plant and Cell Physiol.* **12,** 881 (1971).
[14] J. F. THOMPSON, *Ann. Rev. Plant Physiol.* **18,** 59 (1967).
[15] J. GIOVANELLI and S. H. MUDD. *Biochem. Biophys. Res. Commun.* **25,** 366 (1966).
[16] J. GIOVANELLI and S. H. MUDD, *Plant Physiol.* **41,** S-13 (1966).
[17] W. A. DODD and E. A. COSSINS, *Biochim. Biophys. Acta* **201,** 461 (1970).

methionine to a greater extent. It is interesting that the efficiency of the incorporation of radioactive intermediates into methionine parallels the efficiency of ethylene formation. The conversion of homoserine to ethylene in apple tissue has been established.[18] Since methionine is the most efficient precursor of ethylene as compared to homoserine and homocysteine, it is concluded that methionine is closer to the immediate precursor of ethylene than homoserine or homocysteine.

If methionine is synthesized in apple tissues via cystathionine pathway, one would anticipate that homocysteine is converted into methionine and ethylene more efficiently than is homoserine. However, Table 5 shows that even though less total mass of homocysteine was administered to the apple than that of homoserine, the percent conversion into ethylene from homocysteine is not much higher than that from homoserine. We have established that homocysteine is a more efficient precursor of ethylene than homocystine (Table 5). A lower conversion to ethylene was observed if the prepared L-homocysteine was not used immediately. This is understandable since homocysteine is readily oxidized to homocystine in a neutral solution. When homocysteine is freshly prepared according to the method of Duerre and Miller,[19] more than 85% of the radioactivity exists as free thiol as revealed by PC of the maleimide–homocysteine adduct.[20] After incubation for 3 hr with apple tissue, it was found that most of the radioactivity in the extract was homocystine and there was very little radioactive homocysteine as revealed by the PC of the maleimide



SCHEME 1. METHIONINE-SULFUR CYCLE IN RELATION TO THE BIOSYNTHESIS OF ETHYLENE IN APPLE TISSUE.

[18] S. F. YANG and A. H. BAUR, *Qual. Plant. Mater. Veg.* **19**, 201 (1969).
[19] J. A. DUERRE and C. H. MILLER, *Anal. Biochem.* **17**, 310 (1966).
[20] R. ELLIS, *Nature, Lond.* **211**, 1266 (1966).

adducts.[20] These data indicate that exogenously administered homocysteine is readily oxidized in apple tissue to homocystine which is a less effective substrate for synthesis of methionine and ethylene. This rapid oxidation of homocysteine to homocystine in apple tissue may explain why exogenously supplied homocysteine is not a more efficient precursor of ethylene than is homoserine.

CONCLUSION

The data presented above are consistent with the methionine sulfur cycle depicted in Scheme 1. When methionine is degraded to yield ethylene, the methylmercapto group is transferred presumably to a serine derivative to form *S*-methylcysteine (Tables 1 and 2). In this conversion the methyl group and the sulfur atom of methionine are transferred as a unit. In the conversion of *S*-methylcysteine to methionine, sulfur is incorporated preferentially over the methyl group (Table 3). *S*-Methylcysteine is first demethylated to yield cysteine which then donates its sulfur to form methionine presumably through cystathionine and homocysteine. Such a view is supported by the observations that cysteine, homoserine and homocysteine are converted to methionine with homocysteine being the most efficient precursor of ethylene (Table 5). Though homoserine, homocysteine and methionine are all converted into ethylene, methionine is the most efficient precursor, and is therefore closest to the immediate precursor of ethylene (Table 5).

EXPERIMENTAL

Plant materials and chemicals. Apples used were Golden Delicious which were bought from the local market. L-Methionine-U-^{14}C and DL-homoserine-4-^{14}C were products of Schwarz Bioresearch. Amersham Searle supplied methanethiol-^{35}S, L-cysteine-^{35}S, DL-cysteine-3-^{14}C and methyliodide-^{3}H. Methanethiol-^{14}C was purchased from the New England Nuclear Corp. L-Methionine-methyl-^{14}C was obtained from International Chemical and Nuclear Corp. L-Homoserine-4-^{14}C was prepared from DL-homoserine-4-^{14}C with D-amino acid oxidase.[21] *S*-methyl-L-cysteine-^{35}S and -3-^{14}C were synthesized from L-cysteine-^{35}S and 3-^{14}C, respectively, with MeI. *S*-Methyl-L-cysteine-methyl-^{3}H was synthesized from L-cysteine with MeI-^{3}H. Purification of these chemicals was carried out on ChromAR 500 silicic acid sheets using *n*-BuOH–HOAc–H_2O (4:1:4) (BAW). L-Homocysteine-U-^{14}C was prepared from L-methionine-U-^{14}C by heating 10 μCi of methionine in 0·1 ml 48% HI for 48 hr at 30°. This treatment yields the homocysteine thiolactone. After evaporation homocysteine thiolactone was separated from HI and I_2 by paper electrophoresis at pH 6·3 for 30 min at 40 V/cm. After the thiolactone was eluted and concentrated, it was subjected to alkaline hydrolysis in 5 N NaOH.[19] Free thiol groups were determined by preparing the maleimide adduct followed by paper chromatography in BAW.[20] L-Homocystine-U-^{14}C was prepared from L-homocysteine-U-^{14}C by passing an air stream through the homocysteine solution for 4 hr. Completeness of the oxidation was verified by the failure of the radioactivity to form the maleimide adduct.

Feeding of radioactive substrates and gas analysis. Plugs (1 cm in dia. and 2 cm in length) of apple tissue were cut with a cork borer and razor blade. Except for methanethiol experiments, radioactive substrates were dissolved in 50–100 μl of 2% KCl solution, and were introduced into the plug by a vacuum injection technique described previously.[22]

Analyses of total and radioactive CO_2 and ethylene were performed by GC and gas radiochromatography.[22] For the introduction of radioactive methanethiol, the apple plugs were first sealed in 50 ml Erlenmeyer flasks with rubber serum caps. After partial evacuation, the radioactive methanethiol was introduced with a syringe and needle into the gas phase of the flasks. Radioactivity remaining in the gas phase was measured periodically by withdrawing a gas sample and counting the radioactivity in a liquid scintillation counter.

Extraction and isolation of amino acids. At the end of the incubation, the apple plugs were ground and extracted with MeOH–$CHCl_3$–H_2O.[23] Amino acids were purified by adsorption on ion exchange resin (Dowex 50, H^+ form) and elution with 2 N–NH_4OH. Since methionine and *S*-methylcysteine are readily oxidized to the sulfoxides during this process, they were reduced back to thioethers by heating in 0·1% mercaptoethanol for 60 min at 100°. PC was carried out in BAW.

[21] A. MEISTER, *Biochem. Preparat.* **3**, 66 (1953).
[22] A. H. BAUR and S. F. YANG, *Plant Physiol.* **44**, 1347 (1969).
[23] R. L. BIELESKI and R. A. TURNER, *Anal. Biochem.* **17**, 278 (1966).

Identification of cysteine-^{35}S. This amino acid was identified from apple tissue incubated with *S*-methyl-L-cysteine-^{35}S. After extraction, the extracts were mixed with unlabeled cysteine. Its identification was confirmed based on; (a) preparation and paper radiochromatography of the *N*-ethylmaleimide adduct;[20] and (b) oxidation to cysteic acid with H_2O_2–$HClO_4$ followed by electrophoresis at pH 2·5 and 7·0. The radioactive material obtained from apple tissue agreed in all tests with authentic L-cysteine or cysteic acid. Since the oxidation of cysteine and cystine to cysteic acid with H_2O_2–$HClO_4$ is quantitative, this procedure was employed for the estimation of the radioactive cysteine and cystine.

Identification of S-*methylcysteine.* This amino acid was identified in apple tissues incubated with methanethiol-^{14}C or -^{35}S, L-cysteine-^{35}S, or L-methionine-^{35}S or -methyl-^{14}C. After standard extraction, separation and reduction of the amino acids, 1 μmol of unlabeled *S*-methylcysteine was added to the amino acid extract and the mixture was separated by PC on Whatman 3M paper. The area corresponding to *S*-methylcysteine was eluted with 50% ethanol and concentrated under N_2. Further chemical identification was carried out as follows; (a) cochromatography on Backerflex Silica gel 1B thin layer plate and ChromAR 500 paper and paper electrophoresis at pH 2, 6·5 and 11·5; (b) about 2 mμCi of the suspected *S*-methylcysteine was oxidized with 0·1 ml of 1·8% H_2O_2 in 50% HOAc for 1 hr at 40°. This oxidation yielded a radioactive and ninhydrin positive spot with low R_f corresponding to authentic *S*-methylcysteine sulfoxide on paper chromatography. The spot was eluted and reduced with mercaptoethanol. Rechromatography of the reduced product on paper revealed a shift of R_f to the original value agreeing with standard *S*-methylcysteine. All tests confirmed that the radioactive metabolite isolated from apple tissue was *S*-methylcysteine.

Identification of methionine. Radioactive methionine was isolated and identified using the same methods employed for *S*-methylcysteine. The identity of methionine was further confirmed by GLC on a silicone SE-30 column at 112° of the trimethylsilyl derivative which was prepared by heating the isolated radioactive compound with bis(trimethylsilyl)acetamide for 1 hr at 85°. The radioactivity was found in the fraction where the known trimethylsilyl derivative of methionine was eluted.

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