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THE METABOLISM OF METHIONINE IN HIGHER PLANTS:

CATABOLISM OF THE METHYL GROUP BY SEEDLINGS OF VARIOUS SPECIES

W. E. SPLITTSTOESSER* and MENDEL MAZELIS

Department of Food Science and Technology, University of California, Davis

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Abstract—L-Methionine-$^{14}CH_3$ was rapidly metabolized after infiltration into pea, pumpkin, kohlrabi, and sesbania seedlings. In all cases the labeled carton atom was incorporated into the organic acid, neutral sugar, and amino acid fractions prepared from the seedlings after incubation with the radioactive methionine. Up to 4 per cent of the total activity fed was recovered as $^{14}CO_2$ after 24 hr. At this time as much as 30 per cent of the label was found in the organic acid fraction. In sesbania the activity in this fraction was localized in one compound primarily, whereas the other plants appeared to have two predominant products formed. Among the labeled components of the amino acid fraction, methionine, methionine sulfoxide, serine, and methyl methionine sulfonium were identified and were found in every species tested. The formation of methionine sulfoxide appeared to be a physiological process and not an artifact of the isolation procedure. The presence of the methyl sulfonium salt of methionine in every case implies that this compound may have a significant metabolic role in higher plants.

INTRODUCTION

MOST of the studies to date on the metabolism of methionine have been concerned with its capacity to act as a methyl donor. However the conversion of the methyl carbon to CO_2 was found to occur quite readily in rats fed L-methionine-$^{14}CH_3$[1-3].

Kisliuk *et al.*[3] also found that the β-carbon of serine isolated from the livers was highly labeled, and further showed the glucose of the liver glycogen was labeled to a considerable degree particularly in the 1, 2, 5, and 6 carbon atoms. These authors also studied the catabolism of the other carbon atoms of methionine by feeding DL-methionine labeled in the 2, 3, or 4 position. Few studies have been reported of the carbon catabolism of methionine in higher plants except for its role in transmethylation reactions. In this respect it has been shown that the methyl groups of such common plant constituents as choline,[4] betaine,[4] and various alkaloids[5] are derived from the methyl group of methionine. The metabolism of the optical isomers of α- and β-methionine by intact plants of *Nicotiana rustica* has been examined.[6]

The results of an investigation of the distribution and oxidation of the methyl carbon of L-methionine-$^{14}CH_3$ after feeding to seedlings of various species are given in the present report.

* Present address: Dept. of Horticulture, University of Illinois, Urbana. Please address all reprint requests to the second author (M. M.).

[1] C. G. MACKENZIE, J. P. CHANDLER, E. B. KELLER, J. R. ROCHELLE, N. CROSS, D. B. MELVILLE and V. DUVIGNEAUD, *J. Biol. Chem.* **169**, 757 (1949).
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It was found that the methyl carbon is readily converted to $^{14}CO_2$ and is utilized in reactions which lead to the appearance of the radioactive carbon atom in significant amounts in the organic acid, neutral sugar and ethanol-insoluble fractions of extracts of the seedlings of the higher plants examined. In addition, labeled methionine sulfoxide, methyl methionine sulfonium and serine were found in the amino acid fraction.

RESULTS

Most of the experiments to be reported were conducted using sesbania seedlings (*Sesbania macrocarpa*, Leguminosae) with the roots removed to facilitate greater penetration of the labeled methionine. Table 1 gives a comparison of the distribution of ^{14}C in sesbania seedlings utilized with and without roots. When the roots were removed a greater percentage of the label was found in the organic acid fraction. Other differences were slight. No differences were found in the individual components of the amino acid or organic acid fractions, however, and in the remainder of the work the roots were removed and only the shoots and attached leaves used because of ease of handling.

TABLE 1. DISTRIBUTION OF ^{14}C IN 7-DAY-OLD SESBANIA SEEDLINGS WITH AND WITHOUT ROOTS AFTER INCUBATION WITH L-METHIONINE-$^{14}CH_3$*

Fraction	Intact seedlings		Roots removed	
	c/min	% of total c/min	c/min	% of total c/min
Carbon dioxide	3,700	1·0	3,700	1·2
Amino acids	250,600	68·1	138,900	45·5
Organic acids	53,100	14·5	100,500	32·9
Sugars	2,600	0·7	5,000	1·6
Insoluble residue	57,700	15·7	57,000	18·7
Total ^{14}C	367,700		305,100	

* The incubation time was 6 hr.

Oxidation of the methyl group of methionine to $^{14}CO_2$. Collection of the respired CO_2 showed that a significant amount of the methyl group of methionine was oxidized to CO_2. Figure 1 illustrates that the rate of this oxidation is nearly linear over the first 6 hr in all of the four species tested. After this time, the rate decreases in pea and kohlrabi seedlings while in pumpkin a striking production of $^{14}CO_2$ continues amounting to 3·7 per cent of the administered ^{14}C in 24 hr.

Metabolism of methionine by germinating seedlings. The results of 24-hr feeding experiments to germinating seedlings of the four species examined are summarized in Table 2. Over 95 per cent of the methionine fed was accounted for in each case.

Pumpkin seedlings had an incorporation pattern quite different from the other seedlings. A comparatively larger portion of ^{14}C was oxidized to CO_2 and 30 per cent of the label was incorporated into the insoluble residue fraction. These increases account for the decreased percentage of ^{14}C found in the organic acid fraction of pumpkin. No differences in the pattern of incorporation were found between incubation in light or in the dark.

The variations in the composition of the individual components of the amino acid and sugar fractions in the four plant species are shown in Tables 3 and 4. Several labeled amino acids were found. Methionine and methionine sulfoxide accounted for the largest portion

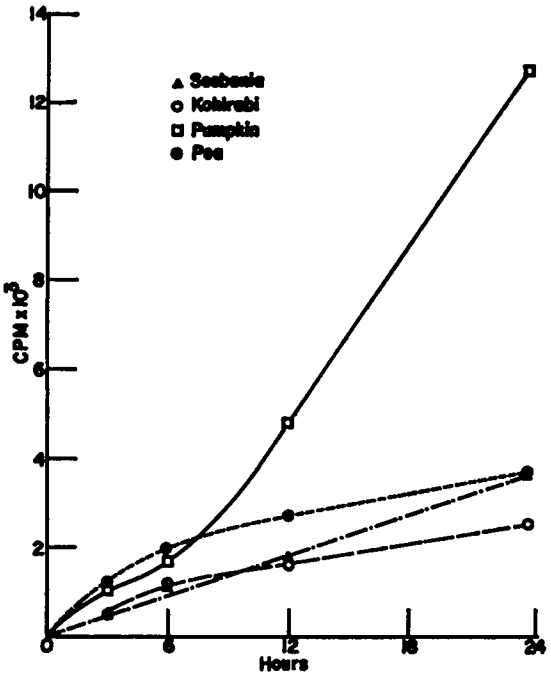

FIG. 1. PRODUCTION OF $^{14}CO_2$ WITH TIME FROM THE RESPIRATION OF L-METHIONINE-$^{14}CH_3$ BY SEEDLINGS OF THE FOUR SPECIES.

TABLE 2. DISTRIBUTION OF ^{14}C WITHIN FRACTIONS OF SEEDLINGS OF THE VARIOUS SPECIES AFTER INCUBATION WITH L-METHIONINE-$^{14}CH_3$*

Fraction	Sesbania (6-day-old)		Pumpkin (6-day-old)		Kohlrabi (7-day-old)		Pea (7-day-old)	
	Total c/min	% of total ^{14}C	Total c/min	% of total ^{14}C	Total c/min	% of total ^{14}C	Total c/min	% of total ^{14}C
Carbon dioxide	3,800	1·9	12,000	3·7	3,500	1·2	3,700	1·2
Lipid	1,400	0·6	6,500	1·8	3,000	1·0	1,400	0·5
Amino acids	133,600	69·2	167,200	48·4	187,400	62·5	137,500	45·0
Organic acids	44,100	22·9	50,100	14·5	80,400	26·8	100,500	32·9
Sugars	1,700	0·7	6,000	1·7	3,600	1·2	5,000	1·6
Insoluble	8,300	4·3	103,700	29·9	21,700	7·2	57,000	18·7
Total ^{14}C incorporated	192,000		346,100		299,600		305,100	

* Incubated 24 hr.

of the total ^{14}C in this fraction. Separation into neutral and basic amino acids and acidic amino acids did not reveal significant amounts of ^{14}C in the latter compounds. Paper chromatography of the acidic amino acid fraction verified that glutamic acid and aspartic acid were not labeled. The amino acid fractions from sesbania and kohlrabi were analyzed for their individual components over a 24 hr period and the results are reported in Tables 5 and 6. Serine and methyl methionine sulfonium were identified in both species as significant constituents of the labeled pool.

Table 3. Distribution of ^{14}C within the amino acid fractions of the various species after 24 hr incubation with L-methionine-$^{14}CH_3$*

Seedling	Methionine	Methionine sulfoxide	Serine	Methyl methionine sulfonium	Others
Sesbania	21	14	49	15	1
Kohlrabi	25	27	35	4	9
Pumpkin	55	3	11	5	26
Pea	24	11	28	17	20

* Expressed as percentage of total radioactivity present in the amino acid fraction.

Table 4. Distribution of ^{14}C within the neutral sugar fractions of the various species after 24 hr incubation with L-methionine-$^{14}CH_3$*

Seedling	Glucose	Fructose	Sucrose	Others
Sesbania	100	—	—	—
Kohlrabi	48	13	—	39
Pumpkin	41	10	25	24
Pea	3	—	3	92

* Expressed as percentage of total radioactivity present in the sugar fraction.

Table 5. Distribution of ^{14}C with time in the amino acid fraction of kohlrabi incubated with L-methionine-$^{14}CH_3$*

Amino acid	Time (hr) 3	6	12	24
Methionine	56	73	51	26
Methionine sulfoxide	41	23	35	39
Serine	3	4	4	20
Methyl methionine sulfonium	—	—	4	7
Others	—	—	6	8

* Expressed as percentage total radioactivity present in the amino acid fraction.

Table 6. Distribution of ^{14}C with time within the amino acid fraction of sesbania incubated with L-methionine-$^{14}CH_3$*

Amino acid	Time (hr) 3	6	12	24
Methionine	80	71	32	25
Methionine sulfoxide	15	24	26	15
Serine	5	5	40	28
Methyl methionine sulfonium	—	—	2	30
Others	—	—	—	2

* Expressed as percentage of total radioactivity present in the amino acid fraction.

In all cases there was a considerable incorporation of the label into organic acids. Paper chromatography showed that this fraction did not contain labeled intermediates of the tricarboxylic acid cycle. In the case of kohlrabi and pea two peaks were found. Sesbania had only one peak, which was identical to one of those in the kohlrabi and pea. DL-Serine-3-^{14}C was fed to sesbania seedlings and produced an organic acid which co-chromatographed with the peak from the methionine experiments. The possibility that it was glyceric acid was eliminated by elution from Dowex 1 with carrier glyceric acid.

Figure 2 illustrates the pattern of incorporation into the four fractions in pea seedlings over 24 hr. After 12 hr only a slight increase occurred in incorporation into sugars and in $^{14}CO_2$ production (see Fig. 1), and the organic acid and insoluble residue activities remained

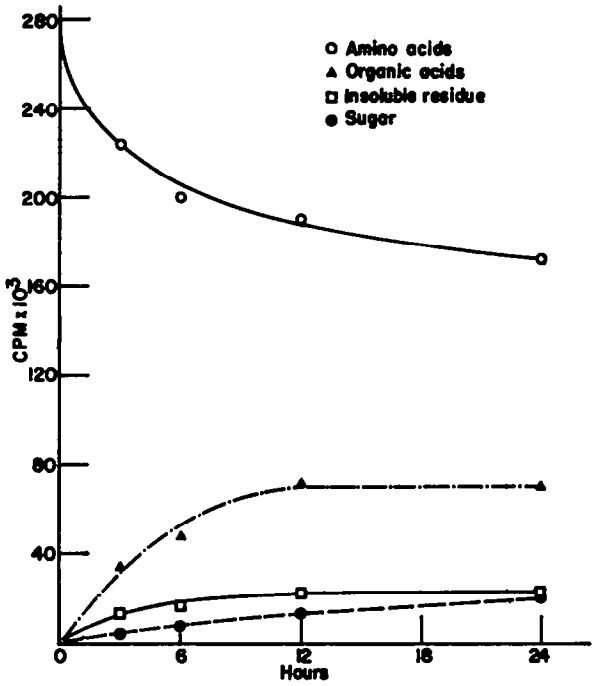

FIG. 2. INCORPORATION OF RADIOACTIVITY FROM L-METHIONINE-$^{14}CH_3$ WITH TIME INTO THE VARIOUS FRACTIONS OF PEA SEEDLINGS.

constant. The pattern of incorporation into sesbania and kohlrabi seedlings revealed a striking similarity, with the exception of a lowered incorporation into the insoluble residue than in pea seedlings.

L-Methionine sulfoxide-methyl-^{14}C was fed to sesbania seedlings. Table 7 shows that in this case a considerable amount of the label was incorporated into the insoluble residue. The results also showed that methionine sulfoxide can be converted to methionine to some extent (Fig. 3). Over 99 per cent of the activity in the amino acid fraction was contained in these two compounds. Distribution of label after feeding methionine-S^{35} to sesbania and kohlrabi seedlings is given in Table 8. A smaller percentage of the label was found in the organic acid fraction compared to the ^{14}C feeding experiments. Methionine, methionine sulfoxide, and methyl methionine sulfonium were identified in the amino acid fractions of both species.

TABLE 7. INCORPORATION OF ^{14}C FROM L-METHIONINE SULFOXIDE-$^{14}CH_3$ INTO VARIOUS FRACTIONS OF SESBANIA SEEDLINGS*

Fraction	Time (hr)			
	3	6	12	24
Amino acids	1118	1093	1055	1035
Insoluble residue	33	58	77	103
All others	1	5	3	2

* Expressed as total c/min × 10^3.

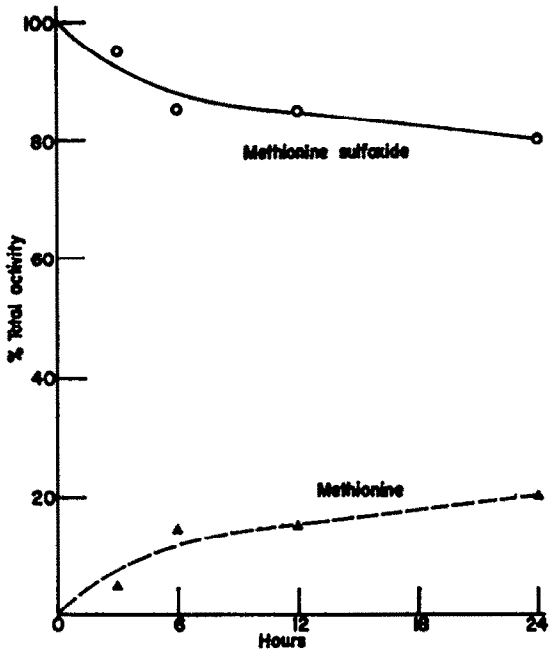

FIG. 3. CONVERSION OF L-METHIONINE SULFOXIDE-$^{14}CH_3$ TO L-METHIONINE WITH TIME BY SESBANIA SEEDLINGS.

TABLE 8. L-METHIONINE ^{35}S METABOLISM BY 6-DAY-OLD SESBANIA AND KOHLRABI SEEDLINGS*

Fraction	Sesbania seedlings		Kohlrabi seedlings	
	c/min × 10^3	% of total incorporated	c/min × 10^3 (thousands)	% of total incorporated ^{35}S
Amino acid	2629	90·0	2842	92·5
Organic acid	101	3·4	100	3·3
Sugars	12	0·4	15	0·4
Insoluble residue	179	6·2	118	3·8
Total ^{35}S Incorporated	2921		3075	

* Incubated 6 hr.

Identification of methyl methionine sulfonium. Methyl methionine sulfonium ^{14}C was identified by co-chromatography in three solvents (see Methods). The methyl methionine sulfonium was eluted from the paper run in the butanol: propionic acid: water solvent system and taken to dryness. It was then reacted with a bacterial sulfonium lyase[7] which catalyses the decomposition of methyl methionine sulfonium to dimethyl sulfide and homoserine. During the reaction the odor of dimethyl sulfide could be detected, and by the end of the reaction the entire label had disappeared. The reaction mixture was passed through a Dowex 50 column and the amino acids eluted and chromatographed on paper. The disappearance of the methyl methionine sulfonium peak and the concomitant appearance of homoserine occurred as predicted. The fact that the label was lost after reaction with the sulfonium lyase indicates that one of the methyl groups at least was derived from methionine-methyl-^{14}C.

Identification of serine. Serine-^{14}C was identified by co-chromatography in three solvents (see Methods). The serine peak was eluted from a strip run in butanol: propionic acid: water and rechromatographed in a phenol: water solvent system. The labeled peak was eluted and acetylated at 0°. The mixture was separated on a Dowex 50 (H^+) column and the non-absorbed N-acetyl-derivative collected. This was co-chromatographed with N-acetylserine 3-^{14}C which was synthesized in the above manner from commercial DL-serine 3-^{14}C. The unknown derivative co-chromatographed identically with the known serine derivative in both the phenol: water and butanol: propionic acid: water solvent systems.

DISCUSSION

Several facts stand out as a result of the feeding experiments described. One of the more obvious ones is the ease with which the methyl carbon of L-methionine is transferred to the various widely different plant fractions. Not all of the reactions involved are due to trans-methylation, since in at least one instance the compound labeled, serine, does not possess a methyl group. It is apparent that although the methyl carbon can be readily oxidized as far as carbon dioxide the distribution in the various fractions cannot be due to fixation of the labeled CO_2 produced since the dicarboxylic organic and amino acids are not labeled.

The labeling patterns in the amino acid fraction of all the species used are of particular interest. The fact that serine is one of the amino acids labeled indicates that there must be a way for the methyl carbon to re-enter the one-carbon pool and contribute to the synthesis of serine. Serine isolated from rats fed L-methionine-$^{14}CH_3$ was labeled almost exclusively in the β-carbon.[3] Presumably the same situation holds in the present case, but the pathways between the methyl group of methionine and the β-carbon of serine have not been clarified to the extent that is known in microbial and mammalian metabolism.

Another interesting observation is the fact that the methyl sulfonium salt of methionine is found as a product in every case. This compound was first isolated from cabbage[8] and has been found as a product of metabolism when radioactive methionine was fed to jack bean,[9] tobacco,[6] and oats.[10] The present work would tend to indicate that this compound is of general distribution in higher plants, and has a metabolic function. Turner and Shapiro[11] have found that many seeds possess a methyl methionine sulfonium-homocysteine trans-methylase.

[7] M. MAZELIS, B. LEVIN and N. MALLINSON, *Biochim. Biophys. Acta* **105**, 106 (1965).
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[10] C. S. SATO, R. U. BYERRUM, P. ALBERSHEIM and J. BONNER, *J. Biol. Chem.* **233**, 128 (1958).
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The question as to whether methionine sulfoxide is a natural metabolite or an artifact of isolation appears to be satisfactorily answered in that it is the former. The results in Table 5 show that in kohlrabi seedlings the amount of sulfoxide relative to the amount of methionine increases with time. If it were an artifact of isolation then the proportion between the two would remain essentially constant. The feeding experiments with methionine sulfoxide in sesbania seedlings show that it can be reduced to the thioether to some extent. An enzyme system in yeast has been found that will carry out this reduction.[12] The sulfoxide can also be utilized as a source of methyl groups for the insoluble residue (Table 8). It does not appear to need reduction to methionine for this purpose. Sato *et al.*[10] have shown that the sulfoxide can act as a donor for pectin in oat seedling sections.

An unusual finding was the relatively large amount of label found in the organic acids of all the species used and its restriction to only a few compounds. The finding that serine-3-^{14}C gave similar results indicates that it is indeed a unique result of C_1 incorporation into the organic acids. The relatively small amount of label in the organic acids found after feeding ^{35}S-labeled methionine shows that it is the methyl carbon that is mobile and not the methylthio group as a unit.

EXPERIMENTAL

Plant Materials

Pea seeds (*Pisum sativum* L. var. Alaska), pumkin seeds (*Curcurbita pepo* L.), kohlrabi seeds (*Brassica caulorapa* Pasq) and sesbania seeds (*Sesbania macrocarpa* Muhl) were soaked 2 hr in distilled water at 25° and then germinated in the dark between moist filter paper for 6–7 days. The roots were removed with a razor blade to facilitate the penetration of the methionine solutions.

Radioactive Materials

L-Methionine-$^{14}CH_3$ was supplied by New England Nuclear Corp. or Volk Radiochemical Co. The stock material was dissolved in distilled water to give a solution of 1 μmole of methionine having 4·5 μc of ^{14}C in 0·05 ml. L-methionine-^{35}S was supplied by Volk already dissolved in water and used as purchased. Paper chromatography revealed a trace of methionine sulfoxide in the samples. Methionine sulfoxide-methyl-^{14}C was synthesized as follows. A 50 per cent excess of H_2O_2 was added to L-methionine-methyl-^{14}C and allowed to react for 4 hr. Catalase was then added to stop the reaction and the entire reaction mixture passed through Dowex 50 (H^+), and the absorbed amino acid was eluted and collected. Methionine sulfoxide-methyl-^{14}C synthesized in this manner contained a trace of unreacted methionine, but there was no methionine sulfone as revealed by paper chromatography in the solvents described below. DL-serine-3-^{14}C was obtained from New England Nuclear Corp.

Incubation Procedure

Duplicate samples of seedlings (five pea or pumpkin seedlings, fifteen kohlrabi or sesbania seedlings) were placed in 1 ml tapered vials containing 0·05 ml of the methionine-methyl-^{14}C solution and then placed in bell jars at room temperature. In 3 hr, the vials were dry and an additional 0·05 ml of water was added to insure complete absorption of the radioactive solution by the seedlings. Six hours after the incubation was initiated, the seedlings were transferred to 5-ml beakers filled with distilled water for the duration of the experiment.

[12] S. BLACK, E. M. HARTE, B. HUDSON and L. WARTOFSKY, *J. Biol. Chem.* **235**, 2910 (1960).

The bell jars were swept with air passed through a 50% KOH scrubber and then Drierite ($CaSO_4$). Respired $^{14}CO_2$ was carried in the air stream and bubbled through 5 ml of 20% KOH in a 50 ml centrifuge tube. The absorbed CO_2 was converted to $BaCO_3$, filtered, and the filter paper counted for radioactivity by a Geiger-Muller tube. The counts were corrected for background and self-absorption.

Analytical Methods

At predetermined times seedlings were removed, rinsed with deionized water to remove any non-absorbed methionine-^{14}C and transferred into 50 ml of boiling 80% ethanol for 3 min. The ethanol was decanted and the tissues were ground with a mortar and pestle. The residues were successively extracted in boiling 80% ethanol, water, and then again with 80% ethanol. The extracts were combined and taken to dryness at 40° under reduced pressure.

The dried ethanol extract was taken up into water and fractionated sequentially on 1×6 cm columns of Dowex 50 (H^+) and Dowex 1 ($HCOO^-$) resins.[13] The amino acid fraction from the Dowex 50 (H^+) column was further fractionated on a Dowex 1 (acetate) column into acidic amino acids, and neutral and basic amino acids. Aliquots of the fractions were taken and assayed for radioactivity.

The components of the fractions were separated by paper chromatography in butanol: propionic acid:water (623:310:437 v/v/v), water-saturated phenol, and *tert*-butanol: methylethylketone:formic acid:water (40:30:15:15).[14] After chromatography, the radioactive components were located by use of a strip counter.

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