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THE RECOVERY OF RADIOACTIVITY IN METHIONINE FROM LABELED *S*-METHYL-L-CYSTEINE IN LEAVES OF *PHASEOLUS VULGARIS*

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Abstract—The metabolism of methyl- and sulfur-labeled *S*-methyl-L-cysteine in excised kidney bean (*Phaseolus vulgaris* L.) leaves was studied. The carbon and hydrogens of the methyl group and the sulfur atom of methylcysteine can be recovered in methionine. The extent of this recovery is markedly influenced by the condition of the bean leaves (healthy, sulfur-deficient, etc.). Experiments with double-labeled methylcysteine showed that the sulfur is not transferred with the methyl group and that the methyl group is not transferred intact. The change in $^{3}H/^{14}C$ between the methylcysteine used and the methionine recovered indicated that two hydrogens of the methyl group of methylcysteine are lost in the transformation. This transfer of the methyl carbon from methylcysteine to methionine is reduced by the use of unlabeled formate. These results indicate that the methyl group of methylcysteine is oxidized to formate and then incorporated into the methyl group of methionine. These results are consistent with a degradation of methylcysteine to methyl mercaptan that is further oxidized to formate. No evidence for the transfer of a methyl group or a thiomethyl group was obtained.

INTRODUCTION

S-METHYL-L-CYSTEINE and its sulfoxide are present in a number of plants.[1] Furthermore, their occurrence in a relatively high concentration in many of these plants could reflect a significant metabolic function in plants. Methylcysteine could serve as a methyl donor or thiomethyl donor.

In vivo studies with radish leaves using methionine labeled with ^{14}C and/or ^{3}H in the methyl group indicated that methylcysteine is formed by a transfer of a methyl group to cysteine.[2] When preliminary experiments with bean leaves revealed the recovery of ^{14}C of the methyl group of methylcysteine in the methyl group of methionine, the possibility that the methyl group of methylcysteine could act as a source of the methyl group of methionine was considered. This paper documents studies into the mechanism of the carbon transfer.

RESULTS AND DISCUSSION

Methylcysteine labeled in the methyl group or with ^{35}S was given to bean leaves (which have no methylcysteine oxidase).[1] Table 1 presents the recovery of radioactivity in selected uncombined amino acids. The results show that methionine is much more labeled than other amino acids by methyl-labeled methylcysteine. Degradation of methionine to remove the methyl group showed that over 80% of the ^{14}C in methionine was in the methyl group. This result could indicate a transmethylation. In addition, methionine was labeled by ^{35}S-methylcysteine. The recovery of ^{35}S was less than that of ^{14}C, even allowing for the

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[1] J. F. THOMPSON, *Ann. Rev. Plant Physiol.* **18,** 59 (1967).

[2] J. F. THOMPSON and R. K. GERING, *Plant Physiol.* **41,** 1301 (1966).

TABLE 1. RADIOACTIVITY OF UNCOMBINED AMINO ACIDS OF THE KIDNEY BEAN LEAF AFTER ADMINISTRATION OF RADIOACTIVE METHYLCYSTEINE

Amino Acid	dis/min ^{14}C-Methylcysteine†
Methionine	110,000
Threonine	10,200
Serine	7170
Homoserine	8980
Aspartic acid	1360
Glutamic acid	1820
Glycine	1360
Alanine	3520

* 1μc of methyl-labeled methylcysteine or 1 μc of ^{35}S-methylcysteine, or both together, were fed to kidney bean leaves for 200 min. Results are total counts in sample (corrected for background) after purification of amino acids and separation on two-directional chromatograms.

† ^{35}S-Methylcysteine alone and a mixture of the ^{14}C and ^{35}S compounds gave methionine with 10,500 and 151,000 dis/min respectively.

TABLE 2. THE RECOVERY OF RADIOACTIVITY IN METHIONINE AFTER INCUBATING BEAN LEAVES WITH RADIOACTIVE METHYLCYSTEINE AND UNLABELED HOMOCYSTEINE*

Label in methylcysteine added	Percent of added label recovered in methionine sulfoxide†			^{3}H/^{14}C‡
	^{14}C	^{3}H	^{35}S	
^{14}C	4·2	—	—	—
^{14}C	4·0	—	—	—
^{3}H	—	0·2	—	—
^{3}H	—	0·5	—	—
^{35}S	—	—	0·0	—
^{35}S	—	—	0·0	—
^{14}C + ^{3}H	2·7	1·3	—	1·1
^{14}C + ^{3}H	4 2	1·9	—	1·1

* Bean leaves were pre-incubated with 10^{-2} M homocysteine for 3 hr and incubated with labeled methylcysteine for 4 hr.

† Recovery of labels in methionine sulfoxide was corrected for counts found in zero time controls.

‡ ^{3}H/^{14}C represents the ratio of counts recovered in methionine sulfoxide as ^{3}H and ^{14}C. The ^{3}H/^{14}C ratio in the methylcysteine used was 2·35.

difference in specific activities. The latter consideration argues against a thiomethyl transfer, but does not disprove it.

Later experiments demonstrated that the addition of unlabeled homocysteine increased the labeling of methionine 3- to 4-fold from methyl-labeled methylcysteine. These later results indicate that there is no direct sulfur transfer. Subsequent findings confirm this indication (see below).

Methylcysteine labeled in the methyl group with ^{14}C and ^{3}H and with ^{35}S was given to bean leaves that had been preincubated with homocysteine. The radioactivity recovered in methionine (as the sulfoxide) is presented in Table 2. The results show no measurable ^{35}S in methionine, indicating no transfer of the thiomethyl group. The ratio of ^{3}H to ^{14}C in methionine sulfoxide is less than in the methylcysteine fed (1·1 vs. 2·35), indicating a loss of hydrogens from the carbon during the transfer. However, the recovery of radioactivity in methionine was so low that the quantitative data were of doubtful significance. As the result of several trials to increase the recovery of radioactivity in methionine, it was found that there was a much greater labeling of methionine if the plants were sulfur-deficient and if the pre-incubation time with homocysteine was shortened to 1 hr.

Table 3 presents the results obtained with leaves from sulfur-deficient bean plants. Treatments 1 and 2 show a much greater recovery of ^{14}C in methionine sulfoxide than in the experiment presented in Table 2 (26–29% vs. 4%). Because of the high levels of recovery of label in methionine sulfoxide, it was felt desirable to confirm its identity. The putative methionine sulfoxide spot was excised, eluted, and the eluate heated with mercaptoethanol to reduce methionine sulfoxide to methionine.[3] Chromatography of the labeled product with methionine confirmed the identity of the original methionine sulfoxide spot.

The addition of unlabeled formaldehyde and formate (treatments 3–6) markedly decreased the label transferred to methionine. This is to be expected if the methyl group is oxidized to formaldehyde or formate. This result is consistent with the decrease in $^{3}H/^{14}C$

TABLE 3. THE RECOVERY OF RADIOACTIVITY IN METHIONINE AFTER INCUBATING SULFUR-DEFICIENT BEAN LEAVES WITH RADIOACTIVE METHYLCYSTEINE AND UNLABELED HOMOCYSTEINE AND WITH OTHER UNLABELED SUBSTANCES*

Treatment	Label in methylcysteine added	Extra unlabeled substance (10^{-2} M) added during 1 hr pre-incubation	Per cent of added label recovered in methionine sulfoxide†		$^{3}H/^{14}C$‡
1	Methyl group-^{14}C	—	28·9		
2	Methyl group-^{14}C	—	26·3		
3	Methyl group-^{14}C	Formaldehyde	6·0		
4	Methyl group-^{14}C	Formaldehyde	3·9		
5	Methyl group-^{14}C	Sodium formate	6·2		
6	Methyl group-^{14}C	Sodium formate	10·1		
7	Methyl group-^{3}H	—	4·4		
8	Methyl group-^{3}H	—	4·1		
9	^{35}S	—	0·7		
10	^{35}S	—	0·5		
11	^{35}S	Cysteine	0·0		
12	^{35}S	Cysteine	0·1		
			^{14}C	^{3}H	
13	Methyl group-^{14}C + ^{3}H	—	14·7	4·1	12
14	Methyl group-^{14}C + ^{3}H	—	11·0	3·6	15

* Sulfur-deficient bean leaves were pre-incubated with 10^{-2} M homocysteine for 1 hr and then incubated (without added homocysteine) for 4 hr.

† Recovery of label in methionine sulfoxide was corrected for counts found in zero time controls.

‡ $^{3}H/^{14}C$ represents the ratio of counts recovered in methionine sulfoxide as ^{3}H and ^{14}C. The $^{3}H/^{14}C$ in methylcysteine used was 45.

[3] R. C. DONEY and J. F. THOMPSON, *Biochim. Biophys. Acta* **124**, 39 (1966).

shown in Table 2. Since formaldehyde and formate can act as one-carbon precursors of the methyl group of methionine,[4–7] it appears that the methyl group of methylcysteine is metabolized via formaldehyde and formate en route to methionine.

The recovery of ^3H in methionine sulfoxide (4·1–4·4%) is considerably less than recovery of ^{14}C (26·3–28·9%), indicating that some of the ^3H is lost in the transfer of the ^{14}C from methylcysteine.

Further evidence against transmethylation is the reduction of radioactivity in methionine in the presence of unlabeled formate, and the lower ratio of ^3H to ^{14}C (av. = 13·5) in methionine as compared to a value of 45 in methylcysteine given. The lower radioactivity in methionine caused by formate implies that formate or a close metabolic product is an intermediate in the transfer of the carbon from the methyl group of methylcysteine to that of methionine. The decrease in $^3H/^{14}C$ to one-third its initial value (showing a loss of 2 of the 3 hydrogens of the methyl group) indicates that formate may be an intermediate.

Very little sulfur (0·5–0·7%) from methylcysteine was recovered in methionine, and the addition of 10^{-2} M cysteine eliminated all ^{35}S in methionine. Presumably, the sulfur is lost from methylcysteine and returns to methionine via cysteine.

The ^3H to ^{14}C ratio in the unmetabolized methylcysteine from treatments 13 and 14 was 63 and 64, in contrast to an initial ratio of 45. The increase in ratio may be due to an isotope effect where the methyl groups with ^3H atoms are not metabolized as readily as those with ^1H atoms.

GENERAL DISCUSSION

These results have shown that in bean leaves the carbon and hydrogen of the methyl group of methylcysteine can be transferred to the methyl group of methionine. However, the methyl group is not transferred intact, but apparently is oxidized to formaldehyde and/or formate. Presumably, the latter compounds are incorporated into the methionine methyl group through N^5-methyl tetrahydrofolate.[8,9] The evidence that the methyl group of methylcysteine is oxidized to a one-carbon intermediate is based on the following evidence: (1) a reduction in the transfer of the methyl carbon of methylcysteine by formaldehyde or formate; (2) a decrease in the ratio of ^3H to ^{14}C in the transfer from methylcysteine to methionine; or (3) the higher conversion of the methyl carbon from methylcysteine to methionine in the presence of unlabeled homocysteine.

Some of the mechanisms involved in the transfer of the methyl carbon of methylcysteine to the methyl group of methionine can be considered. A methylcysteine-degrading enzyme has not been shown in kidney beans. *Albizzia lophantha*[10,11] and *Neurospora crassa*[12] have an enzyme that degrades methylcysteine to methyl mercaptan, and it is not unreasonable that kidney bean should have the same enzyme.

$$\text{methylcysteine} \rightarrow CH_3SH + NH_3 + \text{pyruvate}$$

[4] W. SAKAMI and A. D. WELCH, *J. Biol. Chem.* **187,** 379 (1950).
[5] A. NAKAO and D. M. GREENBURG, *J. Am. Chem. Soc.* **77,** 6715 (1955).
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[10] R. GMELIN, G HASENMAIER and G. STRAUSS, *Z. Naturforsch.* **120,** 687 (1957).
[11] S. SCHWIMMER and A. KJAER, *Biochim. Biophys. Acta* **42,** 316 (1960).
[12] D. P. MOORE and J. F. THOMPSON, *Biochem. Biophys. Res. Commun.* **28,** 474 (1967).

The methyl mercaptan so formed is presumably oxidized to formate and sulfate in a manner analogous to methylcysteine oxidation in animals.[13]

$$CH_3SH \rightarrow HCOOH + SO_4^{2-}$$

Evidence that two of the three hydrogens of the methyl group of methylcysteine are lost in the transfer to the methyl group of methionine indicates that the oxidation proceeds to the formate stage. This is further confirmed by the fact that unlabeled formate reduces the transfer. The reduction in this transfer by formaldehyde indicates that the formate is reduced to formaldehyde. Probably formaldehyde is incorporated into the tetrahydrofolate system for the synthesis of N^5-methyl tetrahydrofolate,[8,9] the methyl donor in methionine biosynthesis.

The initial impetus for this study was to determine whether methylcysteine might serve as a methyl donor or a thiomethyl donor in methionine synthesis in the kidney bean. The results show that methylcysteine is not a methyl donor or a thiomethyl donor, although a small proportion of direct transfer cannot be ruled out. The methyl group of methylcysteine probably is metabolized to formate which can be incorporated into the methyl group of methionine. It has been shown that in sulfur-deficient beans, the methyl group of methylcysteine can be a significant factor in the formation of the methyl group of methionine. Whether this would be true in a normal plant is questionable. The results show that the sulfur of methylcysteine can serve as a precursor of methionine sulfur, but the pathway is probably not direct or important in the overall metabolism of the plant unless the plant is sulfur-deficient.

EXPERIMENTAL

Preparation of Radioactive Methylcysteine

Radioactive methylcysteine labeled in the methyl group was prepared from L-cysteine and ^{14}C-methyl iodide (4·95 μc/μmole) or ^{3}H-methyliodide (74 μc/μmole) in the presence of Na_2CO_3 as previously outlined.[3] Likewise, ^{35}*S*-labeled methylcysteine was prepared from ^{35}*S*-cysteine (2 μc/μmole).

Infiltration and Incubation Procedures

Bean leaves were excised from the plant and the entire petiole immediately immersed in H_2O. Leaves that remained turgid for 16 hr were used. Unlabeled and labeled compounds were then added to the bean leaf tissue via the transpiration stream. A glass collar[2] was used around the petiole to prevent wilting of the leaf during the uptake of unlabeled and labeled compounds. Homocysteine and other compounds (Table 3) were preincubated for 1 hr prior to incubation with labeled methylcysteine (4 hr). At the end of the incubation period, the leaves were cut up and amino acids were thoroughly extracted in 95% EtOH for 24 hr, and then 80% EtOH.

Purification and Isolation of Amino Acids

In one experiment (Table 1), the uncombined amino acid complement of bean leaves was examined. In this work, the amino acids were purified on ion exchange resins;[14] chromatographed two-directionally.[15] The amino acids were located by fluorescence[16] after heating at 110–120° with napthoquinone-4-sulfonate for 3 min.

Because methionine is so readily oxidized to the sulfoxide, it was recovered as such. Methionine sulfoxide, methionine, and methylcysteine were purified as before[3] and separated by paper chromatography with MeOH–pyridine–H_2O (85:15:4, by vol.).[3]

Degradation of Methionine Sulfoxide

Methionine sulfoxide was degraded by refluxing with HI. The MeI so produced was trapped in NMe_3.[17]

[13] E. J. KUCHINSKAS, *Arch. Biochem. Biophys.* **112**, 605, 610 (1965).
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Counting Procedures

After the amino acids were located on the chromatograms, the areas corresponding to individual amino acids were excised, cut into pieces approximately 0·5 × 0·5 cm. The pieces from one area were placed in a standard scintillation vial with 5 ml H_2O and allowed to stand for 1 hr with occasional shaking. Then 15 ml of Bray's solution[18] were added and radioactivity determined by liquid scintillation. Counting efficiency was determined by external standardization.

Reduction of Methionine Sulfoxide

Methionine sulfoxide was reduced to methionine by heating it in a solution of 10^{-2} M mercaptoethanol for 15 min at 120°.[3]

[18] G. A. BRAY, *Anal. Biochem.* **1**, 279 (1960).