Biosynthesis of S-methylmethionine in the jack bean

McRORIE *et al.*[1] showed S-methylmethionine to be a natural constituent of several plant tissues. Several groups of workers have studied the metabolism of S-methylmethionine in animals[2], microorganisms[3] and plants[4].

We have shown that S-methylmethionine is a major metabolite of methionine in young jack-bean seedlings[5] and the present study demonstrates its synthesis from methionine and S-adenosylmethionine in cell-free extracts of jack-bean roots. The reaction presumably involves the transfer of a methyl group from S-adenosylmethionine to the sulfur of methionine as shown in the following equation:

Methionine + S-adenosylmethionine $\rightleftharpoons$ S-methylmethionine + (S-adenosylhomocysteine)

When jack-bean seedlings are grown in the presence of labeled methionine 40–80 % of the non-protein radioactivity is found in a single basic amino acid. This amino acid has been isolated and identified as S-methylmethionine.

S-methylmethionine was isolated from plant extracts by a method, which will be described in a later paper, involving chromatography on the ammonium forms of Dowex 50 and Amberlite CG 120. The radioactive compound isolated from plants grown on L-[^{35}S]methionine or DL-[2-^{14}C]methionine was shown to be S-methylmethionine by comparison with authentic material in several chromatography solvents including the two-dimensional system of CALVIN AND BENSON[6], and by identification of the radioactive products of degradative procedures (Table I). When the roots and the remainder of the plant were extracted separately, it was found that the labeled S-methylmethionine was almost entirely restricted to the roots (Table II).

Because of the localization of S-methylmethionine in the roots of intact seedlings, root tissue was used as a source of enzyme in the study of S-methylmethionine synthesis.

Cell-free extracts were prepared by grinding jack-bean hypocotyls or roots of young seedlings with 2.5 parts of buffer (0.1 *M* potassium phosphate, pH 7.2, 0.008 *M*

TABLE I

Treatment	*Labeled product*	*Identification procedure*
6 *N* HCl, 100°, 7 h	[^{14}C]methionine	Paper chromatography
1 *N* NaOH, 100°, 3 h	[^{14}C]homoserine	Paper chromatography
KBH_4	[^{14}C]aminobutyric acid	Paper chromatography
10 *N* KOH, 100°, 20 min	[^{35}S]dimethyl sulfide	Gas chromatography

TABLE II

Green plant extract (without roots)	*Root extract*		
Total counts/min	*Total counts/min*	*% Activity in basic amino acid fraction*	*Basic amino acid activity in S-methylmethionine peak of Amberlite CG-120*
$2.46 \cdot 10^4$	$1.92 \cdot 10^5$	83.3	—
—	$2.12 \cdot 10^6$	39.4	97.5

glutathione, 30% polyethylene glycol with the omissions noted in Table III) and spinning the homogenate in a Lourdes Refrigerated Centrifuge. The supernatant solution was incubated with labeled methionine under the conditions described in Table III. The basic amino acids were isolated from the deproteinized incubation mixtures by chromatography on hydrogen Dowex 50 using HCl as the eluant.

TABLE III

Homogenized and incubation medium	*% Isotope incorporated into S-methylmethionine*
Complete	5.13
— S-Adenosylmethionine	0.62
Complete	4.80
— Glutathione	1.49
Complete	7.22
— Polyethylene glycol	0.74

Complete medium contains 0.07 *M* potassium phosphate, pH 7.2, 0.006 *M* glutathione, 21% polyethylene glycol-400 (Fisher), 0.0006 *M* S-adenosylmethionine, 0.0002 *M* methionine. Each incubation vessel has a total volume of 3.3 ml and contains 1 ml root extract and $2–5 \cdot 10^5$ counts/min [^{14}C-*Me*]methionine. Vessels were incubated at 30° for 4 h.

S-methylmethionine was shown to be the only radioactive compound in this fraction by paper chromatography and electrophoresis and by chromatography on ammonium Amberlite CG 120 (Table II). The requirements for S-methylmethionine synthesis in a cell-free system are given in Table III. The requirement for S-adenosylmethionine at low concentration is thought to indicate the participation of this substance as a reactant as depicted in the equation above. Inclusion of polyethylene glycol and glutathione in the homogenization and incubation medium yields greater activity. The function of polyethylene glycol is not clear, but similar polymers have been shown to stabilize aldolase and triose phosphate dehydrogenase in crude plant extracts[7]. Incorporation of variously labeled methionine into S-methylmethionine by cell-free extracts is shown in Table IV. The observation that [^{35}S]- and [^{14}C-*Me*]methionine, which both have the L-configuration, are incorporated to roughly twice the extent of DL-[2-^{14}C]methionine suggests that this enzyme is specific for L-methionine.

These observations represent the first example of the biosynthesis of a naturally occurring sulfonium compound other than S-adenosylmethionine; should studies with more highly purified enzyme preparations demonstrate reversibility, this reaction could permit S-methylmethionine to function as a biological reservoir of active methyl groups.

TABLE IV

Labeled compound	*% Incorporation*
L-[^{35}S]Methionine	10.3
DL-[2-^{14}C]Methionine	4.1
L-[^{14}C-*Me*]Methionine	8.0

Conditions similar to Table III except each vessel contained 3 ml root extract. Incubation 5 h at 30°.

This investigation was supported in part by Research Grant RG-5509 from the U.S. Public Health Service.

Radioisotope Service, Veterans Administration Hospital and Department of Biochemistry, Duke University Medical Center, Durham, N.C. (U.S.A.)

RONALD C. GREENE
NANCY B. DAVIS

[1] R. A. MCRORIE, G. L. SUTHERLAND, M. S. LEWIS, A. D. BARTON, M. R. GLAZENER AND W. SHIVE, *J. Am. Chem. Soc.*, 76 (1954) 115.
[2] J. A. STEKOL, in W. D. MCELROY AND B. GLASS, *Amino Acid Metabolism*, Johns Hopkins University Press, Baltimore, Md., 1955, p. 509.
[3] S. K. SHAPIRO, *J. Bacteriol.*, 72 (1956) 730.
[4] J. E. TURNER, *Federation Proc.*, 19 (1960) 7.
[5] R. C. GREENE, *Federation Proc.*, 19 (1960) 7.
[6] M. CALVIN AND A. A. BENSON, *Science*, 109 (1949) 140.
[7] R. H. HAGEMAN AND E. R. WAYGOOD, *Plant Physiol.*, 34 (1959) 396.

Received July 4th, 1960

Biochim. Biophys. Acta, 43 (1960) 360–362

Transfer of radioactivity from the prelabelled cell debris to the microsomal protein

The study of HENDLER[1], showing that mince of hen oviduct incorporates [^{14}C]amino acids into protein but homogenates of the same tissue do not incorporate the amino acids, suggested to us the possibility of the involvement of a factor similar to the prelabelled cell debris concerned in the incorporation of [^{14}C]amino acids into protein in silkworms' silk glands.

In fact, as shown in our previous paper[2], the ability to incorporate [1-^{14}C]glycine into the protein fraction of silk glands was decreased to about one hundredth by the homogenation of the glands.

Studies carried out using subcellular fractionation and incubation of silk glands essentially according to the methods of SHIMURA[3] for the incorporation of ^{14}C-labelled precursors into microsomal protein, with cell debris as the prelabelled source of ^{14}C, showed a marked increase in the radioactivity incorporated into protein. When [1-^{14}C]glycine was used as the source of radioactivity, the relative specific activity of the microsomal protein was less than 0.0001 %, whereas in the case of cell debris as precursors the value was generally around 20 %.

The complete reaction mixture contained approximately 5.8 mg E_1 protein, 7.8 mg E_2 protein and 3.2 mg E_3 protein; 16 μmoles ATP; 20 μmoles phosphocreatin; 8 μmoles $MgCl_2$; 120 μmoles KCl; 100 μmoles Tris–HCl (pH 8.0); 800 μmoles sucrose; 1.0 mg creatine kinase; 0.5 μmole GTP; and 19 amino acids (8 μmoles DL-alanine, 4 μmoles L-serine, 0.8 μmole L-tyrosine, 2 μmoles of DL-valine and DL-threonine, 1 μmole of L-aspartic acid, L-asparagine, L-phenylalanine and L-glutamic acid, 0.5 μmole of L-isoleucine, L-histidine, L-leucine, L-proline and L-arginine, 0.25 μmole of L-glutamine, L-cystine, L-methionine, L-tryptophan and L-lysine).

[2-^{14}C]Glycine-labelled silk glands cell debris was obtained by the following method: 15 min after the injection of 2 μC [2-^{14}C]glycine *per capita*, the silk glands

Abbreviations: ATP, adenosine triphosphate; GTP, guanosine triphosphate; Tris, Tris(hydroxymethyl)aminomethane.