S-Adenosylmethionine formation by barley extracts*

The finding that (—)-S-adenosyl-L-methionine can function as a methyl donor in the enzymic formation of N-methyltyramine, hordenine, and gramine, while L-methionine, L-methionine sulfoxide, and S-methyl-L-methionine are inactive in the same plant-enzyme systems[1] strongly suggests that AMe is an intermediate on the natural pathway of transmethylation in these plants. Since the presence of AMe has not been reported in higher plants, it was important to demonstrate that this compound may indeed be formed by plants. The present paper reports such a demonstration.

L-[*Me*-^{14}C]methionine was incubated with extracts of barley seedlings in the presence of $MgCl_2$, KCl, adenosine-5′-triphosphate and a small pool of AMe. As is shown in Table I, the AMe fraction contained radioactivity only after incubation with radioactive methionine and enzyme. L-methionine sulfoxide did not replace methionine.

TABLE I

FORMATION OF S-ADENOSYLMETHIONINE BY BARLEY EXTRACTS

Substrate	*Counts/min in AMe fraction*	
	Minus enzyme	*Plus enzyme*
L-[*Me*-^{14}C]methionine	22	480
L-[*Me*-^{14}C]methionine sulfoxide	23	32

Each vessel contained in μmoles: tris(hydroxymethyl)aminomethane, pH 7.6, 100; KCl, 100; $MgCl_2$, 50; adenosine-5′-triphosphate, 13.7; (—)-S-adenosyl-L-methionine[2], 1.0; either L-[*Me*-^{14}C]-methionine, 0.11 ($103 \cdot 10^3$ counts/min) or L-[*Me*-^{14}C]methionine sulfoxide (synthesized by treatment of L-[*Me*-^{14}C]methionine with H_2O_2), 0.2 ($127 \cdot 10^3$ counts/min). Vol., 0.50 ml. The enzyme was prepared by grinding 6-day-old barley seedlings with sand and 1.5 vol. (w/v) potassium phosphate buffer, pH 7.0, 0.02 *M*. The suspension was filtered through a plug of glass wool and 1.0 ml of the filtrate added to the reaction mixtures as indicated. Incubation was at 24° for 6 h. After incubation, 0.18 ml 30% $HClO_4$ was added to all vessels and enzyme to "minus enzyme" vessels. 7.5 μmoles carrier L-methionine or methionine sulfoxide were then added. Aliquots of the deproteinized supernatant fluid were neutralized and chromatographed on columns of Amberlite IRC-50 (XE-64), 6 × 1 cm, buffered at pH 7.0[3]. Aliquots of the fractions containing AMe were counted in a thin-window, gas-flow counter.

The AMe fraction was lyophilized and chromatographed again on IRC-50 (XE-64) buffered at pH 7.6 with ammonium phosphate. Under these conditions, AMe is eluted with dilute pH 7.6 buffer after a lag and over a range of several column volumes. It was found that the radioactivity was eluted in a manner parallel to the AMe as judged by its u.v. absorption.

The AMe fractions were combined, passed over a column of Dowex-1 formate and lyophilized. The residue was dissolved in water, the solution brought to pH 5 by the addition of formic acid and heated at 100° for 20 min to decompose the AMe to 5′-methylthioadenosine and homoserine (or homoserine lactone)[4,5]. A paper chromatograph of a concentrate of this solution showed that essentially all the radioactivity was present in the 5′-methylthioadenosine.

A similar series of experiments was carried out using L-[2-^{14}C]methionine.

Abbreviation: AMe, (–)-S-adenosyl-L-methionine.

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Again radioactivity was incorporated into AMe as judged by its behavior on the two ion-exchange columns. Decomposition was performed as above, the resulting radioactive material was desalted by adsorption on Dowex-1 (OH^-) followed by elution with HCl[6] and was identified by paper chromatography as homoserine. In a third set of experiments, L-[1-^{14}C]methionine was shown also to be incorporated into AMe which on decomposition gave rise to radioactive homoserine.

To confirm and extend these findings, some of the [*Me*-^{14}C]AMe was subjected to treatment with a partially purified yeast enzyme which cleaves AMe to 5'-methylthioadenosine and to α-amino-γ-butyrolactone[3]. The reaction was allowed to proceed until 90% of the AMe was decomposed. The specific activities of the initial AMe and the 5'-methylthioadenosine which was formed were the same within experimental error (15,100 and 16,000 counts/min/μmole respectively). Because of the known substrate specificity of this enzyme, these results confirm not only that the radioactivity was in S-adenosylmethionine[3] but clearly establish the configuration at the asymmetric sulfur and α-amino-carbon atoms of the AMe as—and L[3,7].

The facts that AMe is formed by barley and that it is used by this plant and by millet in a group of alkaloid-forming transmethylations suggests that this compound is the predominant methyl donor in plants as it is known to be in animals and microorganisms.

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Inhibition of yeast carboxylase by acetaldehyde

It has been known for some time, in fact since the description of carboxylase by NEUBERG[1], that the velocity of the reaction

$$\text{pyruvate} \longrightarrow \text{acetaldehyde} + CO_2$$

catalyzed by yeast carboxylase quickly decreases even in the presence of an excess of pyruvate[1,2]. The deviation of the curve of the CO_2 evolution from the straight line to be expected at zero-order conditions has generally been attributed to inhibition of the enzyme by the acetaldehyde formed[1–5], especially as the enzyme is relatively stable during incubation without substrate[2,3,5]. There are, however, to our knowledge