Phytochemistry, 1968, Vol. 7, pp. 2143 to 2145. Pergamon Press. Printed in England

BIOSYNTHESIS OF *S*-ADENOSYLMETHIONINE IN GERMINATING PEA SEEDS

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(*Received* 17 *April* 1968)

Abstract—This communication reports a quantitative study of levels of *S*-adenosylmethionine and *S*-adenosylhomocysteine in pea seeds during the first 3 days of germination. There is net synthesis of both compounds during this period. The *S*-adenosylmethionine, which is in an active state of turnover, is formed from activation of methionine. Isotopic studies indicate that other recognized pathways of *S*-adenosylmethionine biosynthesis are unimportant in germinating pea seedlings.

INTRODUCTION

RECENT work[1-3] with mammalian and plant tissues and with micro-organisms has directed attention to the importance of *S*-adenosylmethionine (SAM) as a methyl donor. However, comparatively little is known of the levels of SAM in higher plant tissues and there have been no reports regarding the pathways for its biosynthesis in metabolically active plant tissues. This note reports some results of studies in which SAM levels have been measured and isotopic tracers used to elucidate the pathway of biosynthesis of this compound in germinating pea seeds.

RESULTS AND DISCUSSION

In view of the close metabolic relationship between *S*-adenosylmethionine and *S*-adenosylhomocysteine (SAHC) (equation 1) it is necessary to account for the concentrations of both of these compounds if an estimate of net synthesis of SAM is to be made.

$$\text{SAM} + \text{methyl acceptor} \rightarrow \text{methylated product} + \text{SAHC} \qquad (1)$$

The experimental results (Fig. 1) show that after 24 hr the level of SAM in germinating pea seeds reaches a maximum. It is of interest to note that this value is within the range of values reported for other tissues (Table 1) when expressed on the basis of fresh weight.

The considerable variation encountered in the levels of SAM and SAHC does not appear to be inherent in the method used since recoveries of authentic SAM and SAHC, subjected to the experimental procedure, were within the range 95–106 per cent. It therefore appears likely that the observed variation is due to variability within the tissue used. Such variation may be ascribed to variable rates of inbibition of water by the dry seeds which would affect the physiological condition of the sampled tissue. It is clear that there is net synthesis of both

[1] G. L. CANTONI, in *Transmethylation and Methionine Biosynthesis* (edited by S. K. SHAPIRO and F. SCHLENK), p. 21, Univ. of Chicago Press, Chicago (1965).
[2] S. K. SHAPIRO, in *Transmethylation and Methionine Biosynthesis* (edited by S. K. SHAPIRO and F. SCHLENK), p. 200, Univ. of Chicago Press, Chicago (1965).
[3] H. KAUSS and W. Z. HASSID, *J. Biol. Chem.* **242**, 15, 3449 (1967).

SAM and SAHC as germination proceeds. Apparently germinating peas are actively synthesizing both compounds and therefore provide an opportunity to examine the biosynthetic pathway involved.

A number of different mechanisms for the biosynthesis of SAM have been demonstrated in various organisms. Several of these involve the methylation of SAHC but do not appear to be applicable in germinating pea seeds, since the pool of SAHC is very small initially and

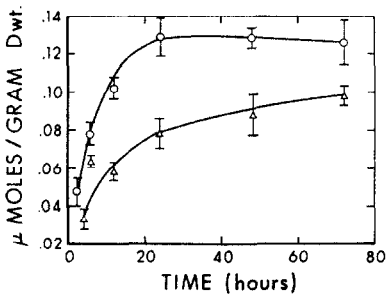

FIG. 1. CHANGES IN THE SAM AND SAHC CONTENT OF PEA SEEDS DURING GERMINATION AT 25°. SAM (○—○) AND SAHC (△—△) WERE ASSAYED SPECTROPHOTOMETRICALLY AS DESCRIBED IN THE TEXT. The vertical lines indicate the standard error of the mean, each data point representing the mean of at least six separate determinations.

TABLE 1. QUANTITIES OF *S*-ADENOSYLMETHIONINE IN DIFFERENT TISSUES

Source	SAM μmoles/g fresh wt.
Candida utilis	0·9–1·5*
Saccharomyces cerevisiae	0·3–0·8*
Rat tissues†	
liver	0·07
heart	0·03
kidney	0·16
muscle	0·08
Germinating pea seeds	0·04

* Data from cultures grown without methionine supplement.[7]
† Data from Pansuwana.[8]

so cannot account for the net synthesis of SAM. Other mechanisms for SAM synthesis not involving the participation of SAHC have been shown. For example Schlenk and Ehninger[4] showed that in the yeast *Candida utilis*, SAM was formed from methylthioadenosine (equation 2) and Davies[5] showed that SAM in turnip storage tissue was synthesized from ATP and methionine (equation 3). The SAM formed in this latter tissue was not metabolically active.

$$\text{Methylthioadenosine} + \text{homoserine} \rightarrow \text{SAM} \quad (2)$$

$$\text{Methionine} + \text{ATP} \rightarrow \text{SAM} + \text{pyrophosphate} + P_i \quad (3)$$

In order to determine the pathway of SAM synthesis in germinating pea seeds, feeding experiments were conducted using tissue slices. In all cases labelled methionine was pulse-fed for 7 hr followed by incubation of the slices in the absence of labelled substrates. When

[4] F. SCHLENK and D. J. EHNINGER, *Arch. Biochem. Biophys.* **106**, 95 (1964).
[5] D. D. DAVIES, *J. Exp. Botany* **17**, 51, 320 (1966).

methionine-^{35}S and methionine (methyl-^{14}C) were supplied, the SAM pool was rapidly labelled and after 7 hr had a specific activity similar to that of the supplied methionine. In the post-pulse period SAM labelled with ^{14}C showed a pronounced decrease in ^{14}C content with a half time of 7 hr. SAM labelled with ^{35}S did not show such a pronounced decrease in radioactivity during the post-pulse period. These data clearly show that the SAM pool is in an active state of turnover during germination. Such turnover may in large part be related to the involvement of SAM in transmethylation reactions. Feeding of uniformly labelled homoserine-^{14}C to 2-day-old germinating pea slices showed little incorporation of ^{14}C into SAM or SAHC. On the basis of these feeding experiments it appears that SAM is formed most readily by the direct activation of methionine (equation 3). Further work on the biosynthesis of SAM and SAHC in these tissues is being carried out and will be the subject of further publications.

EXPERIMENTAL

Preparation of Plant Material

Pea seeds (*Pisum sativum* L. var. Homesteader) were soaked in deionized water for 6 hr at room temperature and then germinated in darkness in moist vermiculite at 25°. Samples of the germinating seeds (10 g fresh wt) were ground in a pestle and mortar at 4° with 5 ml of 1·5 N $HClO_4$. After centrifugation the residue was washed with about 10 ml of ice-cold H_2O. The pH of the combined supernatants was then adjusted to 6·5 by addition of solid $KHCO_3$. A further centrifugation prepared the extract for ion-exchange chromatography.

Chromatographic Method

The method used for isolation and estimation of SAM and SAHC was basically that described by Shapiro and Ehninger.[6] This method involves the sequential elution of the nucleosides from Dowex 50W X8 (100–200 mesh) resin in the Na^+ and H^+ forms using HCl. Both SAHC and SAM were recovered in the 6 N HCl fraction. This fraction was reduced to dryness in a rotary evaporator at 40° and the residues were redissolved in water.

Identification and Assay of Sulphonium Compounds

The sulphonium compounds were authenticated by TLC on silica gel GF (Merck) using *n*-butanol:acetic acid:water, 60:15:25 (by vol.) as the solvent system. Under these conditions SAHC had an R_f of 0·22 and SAM had an R_f value of 0·05. The breakdown products of SAM, namely methyl thioadenosine and homoserine, had R_f values of 0·62 and 0·24 respectively. The separated compounds were localized[9] by their fluorescence under u.v. light and by their reaction with ninhydrin, platinic iodide and aniline xylose sprays. The quantities of SAM and SAHC in the column effluents were assayed spectrophotometrically in a Beckman DB-G spectrophotometer. The molar extinction coefficients[6] for SAM and SAHC in the 6 N acid eluates at 260 nm were taken as Em = 14,700.

Acknowledgements—This investigation was supported by a grant in aid of research to one of us (E. A. C.) from the National Research Council of Canada.

[6] S. K. Shapiro and D. J. Ehninger, *Analyt. Biochem.* **15**, 2, 323 (1966).
[7] F. Schlenk, in *Transmethylation and Methionine Biosynthesis* (edited by S. K. Shapiro and F. Schlenk), p. 48, Univ. of Chicago Press, Chicago (1965).
[8] P. Pansuwana, in *Summary Report Biological and Medical Research Division*, p. 100. Argonne Natl. Lab., 6368 (1961).
[9] M. K. Gaitonde and G. E. Gaull, *Biochem. J.* **102**, 959 (1966).