

THE PARTICIPATION OF *S*-ADENOSYLMETHIONINE IN THE BIOSYNTHESIS OF CAFFEINE IN THE TEA PLANT

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1. Introduction

Previous work on caffeine biosynthesis [1–4] established that caffeine is synthesized from the same precursors utilized for purine and methyl group syntheses in other systems. Of the various methyl donors, methionine is the most effective [1]. However, there have been no reports on the involvement of *S*-adenosylmethionine (SAM) in the biosynthesis of caffeine, in spite of its established importance as a methyl donor in various transmethylation reactions [5,6].

The present paper describes experiments that investigate the participation of SAM in caffeine biosynthesis. Evidence has been obtained that SAM is formed from methionine in tea plants. Tea leaves infiltrated with SAM-methyl- ^{14}C produced caffeine- ^{14}C . These results suggest that SAM is an active methyl donor in caffeine biosynthesis as it is in other systems.

2. Materials and methods

L-Methionine-methyl- ^{14}C (53 mCi/mmole) and *S*-adenosyl-L-methionine-methyl- ^{14}C (50 mCi/mmole) were obtained from Le Commissariat à l'énergie Atomique, France. *Thea sinensis* L. germinated and grown at 20° in normal daylight in a greenhouse for 80 days was used.

2.1. Administration of methionine-methyl- ^{14}C

Excised tea shoots consisting of three leaves were fed with the required quantity of radioactive chemical within 1 hr, and were incubated in distilled water at 20° for various periods.

2.2. Vacuum infiltration of *S*-adenosylmethionine-methyl- ^{14}C

Groups of leaf disks (each 1 g in fresh wt) were infiltrated with 10 μCi of SAM-methyl- ^{14}C in 30 ml of 0.01 M phosphate buffer (pH 5.8) for 2 min under reduced pressure, then were transferred to wet filter papers in petri dishes and incubated at 20° for various periods.

2.3. Extraction, isolation and identification of *S*-adenosylmethionine

Tea shoots were homogenized with 5–6 vol of 1.5 N PCA, an equal weight of washed Polyclar AT and about 1 g of washed sand in a chilled mortar. The homogenate was centrifuged at 9000 *g* for 20 min. The residue was washed with about 10 ml of ice-cold water, then the pH of the combined supernatants was adjusted to 6.5 with solid KHCO_3 . This mixture was centrifuged at 9000 *g* for 20 min.

SAM was isolated from the supernatant fluid by chromatography on a column (5 × 1 cm) of Dowex 50- Na^+ , according to Shapiro and Ehninger [7] as modified by Dodd and Cossins [8]. SAM was recovered in the 6 N HCl fractions [7, 8].

SAM isolated by the Dowex 50- Na^+ column was identified by co-chromatography on silica gel plates (Eastmann Chromagram Sheets 6060) with authentic SAM (Boehringer Mannheim Co.) in 1) *n*-butanol–acetic acid–water (12:3:5) and in 2) 2,4-lutidine–2,4,6-collidine–water (6:5:5) as the solvent systems [9]. Ultraviolet quenching, ninhydrin spray and radioactivity scanning were used for detection.

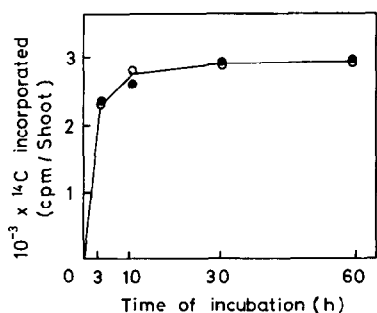


Fig. 1. Incorporation of radioactivity from methionine-methyl-¹⁴C into caffeine by excised tea shoots in light (○—○) and dark (●—●). Two excised tea shoot tips (0.9 g fresh wt) were fed with 2 μ Ci of methionine-methyl-¹⁴C in 0.2 ml of solution within 1 hr, then were incubated in distilled water in 50-ml flasks for various periods. In the dark experiments tea shoots were incubated in a dark cabinet. In the light experiments tea shoots were incubated in normal daylight.

2.4. Caffeine estimation

Caffeine was extracted, isolated and estimated by the method of Grossfeld and Steinhoff [10] as modified by Torii and Ota [11]. Radioactivity of caffeine was determined as follows. After paper electrophoresis of caffeine extracts, darkened spots on the strips due to ultraviolet quenching were marked and cut out. Counting was carried out in a Nuclear-Chicago type 6804 liquid-scintillation counter. The counting solution contained 4 g of PPO and 0.1 g of POPOP in one litre of toluene.

3. Results and discussion

3.1. Incorporation of methionine-methyl-¹⁴C into caffeine by excised tea shoot tips in the light and in darkness

Fig. 1 shows the pattern for incorporation of methionine-methyl-¹⁴C into caffeine by excised tea shoots in the light and in darkness. In both cases, the maximum amount of radioactivity was found at 30 hr. However, obviously the maximum rate of methylation occurred by 3 hr, because almost all the methionine-methyl-¹⁴C supplied was metabolized by 2 hr; no increase in radioactivity after 30 hr was due to exhaustion of the radioactive label supplied. These results indicate that the turnover of methionine is rapid in tea plants.

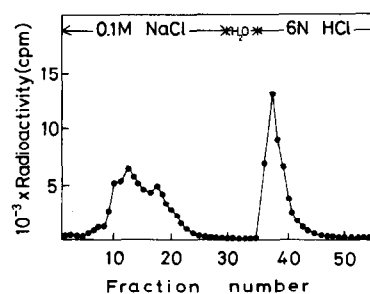


Fig. 2. Ion-exchange chromatography of the acid soluble extract from tea shoot tips (2.4 g fresh wt) labelled with methionine-methyl-¹⁴C for 3 hr. A Dowex 50-Na⁺ column (5 cm X 1 cm) was used. The column was washed with 50 ml of water before applying 0.1 M NaCl. Thirty 10-ml fractions were collected during elution with 0.1 M NaCl. Radioactivities of the fractions were assayed in a Kobe Kogyo PC-26 gas-flow counter. The column was washed with 50 ml of water before applying 6 N HCl. Fractions of 9.0 ml were collected during elution with 6 N HCl. Radioactivities were determined in a Beckman LS-100 scintillation counter. The counting solution and methods used for the radioactive assay were as described by Dodd and Cossins [8].

Light had no appreciable effect on incorporation of the radioactive label into caffeine. This agrees with results reported by Anderson and Gibbs [1].

3.2. Evidence for the formation of S-adenosylmethionine in tea shoots

Formation of SAM from methionine in tea shoots was investigated by feeding tea shoots with methionine-methyl-¹⁴C (5 μ Ci) for 1 hr then incubating them in distilled water for 2 hr. Fig. 2 shows the radioactive profile from ion-exchange chromatography of an acid soluble extract from tea shoots. Radioactivity was recovered in the 6 N HCl fractions. Identification of SAM-¹⁴C was provided by thin-layer chromatography; the R_f value was 0.18 in both systems 1) and 2).

3.3. Incorporation of S-adenosylmethionine-methyl-¹⁴C into caffeine by tea leaf disks

Fig. 3 shows the pattern for incorporation of radioactivity from SAM-methyl-¹⁴C into caffeine by tea leaf disks. Clearly SAM can serve as a precursor of caffeine. Though no attempt has been made to examine the distribution of radioactivity incorporated into caffeine, it appears that SAM, in common with

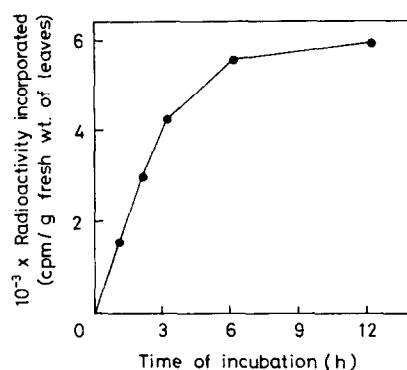


Fig. 3. Incorporation of radioactivity from *S*-adenosylmethionine-methyl-¹⁴C into caffeine by tea leaf disks. Details are given in the text.

other transmethylation reactions, acts as a methyl donor.

These studies clearly show that SAM is synthesized from methionine when considerable synthesis of caffeine is taking place (fig. 1,2). Considering the established role of this compound in a variety of transmethylation reactions [5,6], it is highly likely that this compound has importance in caffeine synthesis also. In fact, the radioactivity of SAM-methyl-¹⁴C is rapidly incorporated into caffeine in tea leaf disks (fig. 3). The methyl transfer from methionine to caffeine precursors may proceed via SAM.

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