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CONVERSION OF 5′-METHYLTHIOADENOSINE TO METHIONINE BY APPLE TISSUE

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Abstract—Conversion of 5′-methylthioadenosine to methionine in apple tissue is demonstrated and discussed in relation to ethylene biosynthesis.

INTRODUCTION

Although methionine has been established as a precursor of ethylene in a number of plant tissues, it is not the immediate precursor [1]. Recently it has been suggested that methionine is converted into *S*-adenosylmethionine prior to its conversion to ethylene in apple tissue [2, 3], and that 5′-methylthioadenosine would be the fragment nucleoside [3]. Since the methionine concentration in apple fruit is extremely low as compared to its ethylene production rate, it has been concluded that the sulfur atom of methionine must be retained and recycled during the continuous synthesis of ethylene [4, 5]. Although 5′-methylthioadenosine has been shown to be converted into methionine prior to its conversion into *S*-adenosylmethionine in yeast [6, 7], there have been no reports concerning metabolism of 5′-methylthioadenosine in higher plants. This paper describes the formation of methionine from 5′-methylthioadenosine-[Me-^{14}C] fed to apple tissue.

RESULTS AND DISCUSSION

Methionine was isolated and identified from apple tissue which had been fed 5′-methylthioadenosine-[Me,^{14}C]. The conversion of 5′-methylthioadenosine to methionine increased with time and after 5 hr incubation about 20% of the radioactivity was recovered as methionine (Fig. 1). A crude apple extract in 80% ethanol was first concentrated *in vacuo* at 45° then reduced with 1% mercaptoethanol (100° for 1 hr). A portion of the extract was cochromatographed with authentic methionine on Whatman 1 paper using *n*-BuOH–HOAc–H_2O (4:1:5). Two major radioactive spots were detected in the region of R_f 0·42 (methionine) and R_f 0·65 (5′-methylthioadenosine). The extract was then passed through a cation exchange resin (Dowex 50, H^+ form) and amino acids eluted with 2N NH_4OH. After concentration, the eluate was

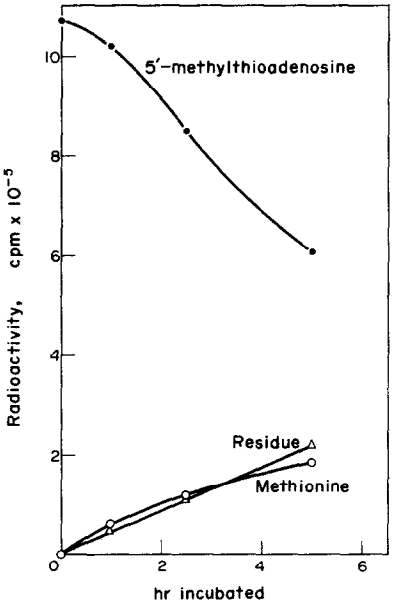

Fig. 1. Time-course for conversion of 5′-methylthioadenosine to methionine in apple tissue. Each apple plug (1 × 2 cm) received 0·5 μCi of 5′-methylthioadenosine-[Me-^{14}C] (50 μCi/μmol). The formation of radioactive methionine was determined after various incubation periods at 20° as described in text and Experimental. The residue refers to radioactivity not extracted by 80% EtOH.

reduced with 1% mercaptoethanol and chromatographed as before. The radioactive spot in the region of R_f 0·42 was identified as methionine according to the following criteria: (a) on paper electrophoresis at pH 1·9, 6·8, and 10·0 and on paper chromatography, the radioactivity migrated the same distance as authentic methionine; (b) upon oxidation with 1·8% H_2O_2 in 50% HOAc for 1 hr at 40°, it yielded a radioactive compound which cochromatographed on paper with methionine sulfoxide (R_f 0·12); (c) when the oxidized material was reduced with 1% mercaptoethanol and chromatographed on paper, the R_f increased to 0·42 where authentic methionine migrated.

A requirement for ATP [2, 3] and pyridoxal phosphate [3, 4, 8] in the conversion of methionine to ethylene has been recently shown. The ATP is suggested to function in the activation of methionine to *S*-adenosylmethionine which then forms a complex with pyridoxal phosphate followed by a concerted γ-elimination reaction yielding CO_2, HCOOH, NH_3, C_2H_4 and 5′-methylthioadenosine [3]. The formation of 5′-methylthioadenosine in this scheme would be consistent with the observation that no volatile sulfur compounds are formed during the conversion of methionine to ethylene by apple tissue [5, 9]. We have shown above that 5′-methylthioadenosine-[Me-^{14}C] is then recycled back to methionine. However, the present results do not indicate whether the methylthio group of 5′-methylthioadenosine is transferred as a unit to form methionine or whether the methyl group is transferred to methionine independent of the sulfur atom. In yeast, it was shown that both the methyl group and the *S* atom of 5′-methylthioadenosine were incorporated into *S*-adenosylmethionine [6]; however, it is not known whether the reaction is a transmethylation or a transmethylthiolation.

EXPERIMENTAL

Plant materials and chemicals. Apples (cv. Golden Delicious) were bought from a local market. *S*-Adenosyl-L-methionine-[Me-^{14}C] was obtained from ICN. 5′-Methylthioadenosine-[Me-^{14}C] was prepared by hydrolysis of *S*-adenosylmethionine-[Me-^{14}C] for 20 min at 100° in dil. HOAc, pH 4·0 [6]. Paper chromatography of the hydrolyzate in *n*-BuOH–HOAc–H_2O (4:1:5) yielded a single radioactive spot in the region of R_f 0·65 which corresponded to authentic 5′-methylthioadenosine. Further verification was made by paper coelectrophoresis at pH 2·1 and 11·0 with authentic sample.

Feeding of radioactive substrate and extraction procedures. Apple plugs (1 cm dia and 2 cm in length) were cut with a cork borer and razor blade and the radioactive substrate in 2% KCl solution was introduced into the plugs by a vacuum injection technique [10]. After incubation, the tissues were ground and extracted with 80% EtOH.

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