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5-METHYLTHIORIBOSE AS A PRECURSOR OF THE CARBON CHAIN OF METHIONINE

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SUMMARY

The enzymatic conversion of 5-methylthioribose to methionine and its deaminated derivatives, 2-keto-4-methylmercaptobutyric acid and 2-hydroxy-4-methylmercaptobutyric acid by cell-free extracts of Enterobacter aerogenes has been demonstrated. ^{14}C-Labeled methionine was isolated from incubation mixtures with 5-methylthio[U-^{14}C]ribose. The carbohydrate part of this compound furnishes at least part, if not all, of the four carbon chain of methionine.

INTRODUCTION

5'-Methylthioadenosine is formed from S-adenosylmethionine by several metabolic routes (1,2), but it does not accumulate in cells because of its rapid hydrolysis to adenine and either 5-methylthioribose or 5-methylthioribose-1-phosphate (3-6). It has been established in a variety of systems that the methylthio moiety of 5'-methylthioadenosine is recycled into methionine (7-11). In Enterobacter aerogenes the second step in this pathway involves a new enzyme, 5-methylthioribose kinase (12). In these reports, it seemed possible that the methylthio group was transferred as a unit to a four carbon acceptor. Experiments with yeast cells provided the first data on the conversion of the ribose carbons of 5'-methylthioadenosine into the four carbon chain of methionine (13). Subsequently, analogous results were reported with rat liver homogenates (14). The results reported here with enzyme extracts of E. aerogenes and 5-methylthio[U-^{14}C]ribose demonstrate that the ribose moiety furnishes part, if not all, of the four carbon chain of methionine.

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MATERIALS AND METHODS

Compounds. 5'-[methyl-^{14}C]Methylthioadenosine was prepared from S-adenosyl-L-[methyl-^{14}C]methionine (15), and 5'-methylthio[U-^{14}C]adenosine was synthesized as described by Borchardt et al (16). 5-[methyl-^{14}C] Methylthioribose and 5-methylthio[U-^{14}C]ribose were obtained by acid hydrolysis of the corresponding 5'-methylthioadenosine preparations (8). The corresponding labeled methylthioribose-1-phosphates were obtained from 5-methylthioribose (1.3×10^6 to 2.0×10^6 cpm/µmol) by incubation with purified 5-methylthioribose kinase (12). The reaction mixtures contained 0.05 M imidazole/HCl (pH 7.3), 0.3 mM labeled 5-methylthioribose, 1.0 mM ATP, 5.0 mM $MgSO_4$, 10.0 mM dithiothreitol, and sufficient purified enzyme to complete the conversion of 5-methylthioribose to the 1-phosphate derivative. The incubation time was 1 h at 37°C. After the addition of 2 vol of ethanol, precipitated salts and proteins were removed by centrifugation. The supernatant fluid was applied to a DEAE Sephadex A-25 column (0.2 x 3.0 cm for micromole quantities) which had been equilibrated with 0.05 M NH_4HCO_3, pH 8.0. 5-Methylthioribose-1-phosphate was quantitatively eluted with 15 ml of 0.075 M NH_4HCO_3, pH 8.0; ADP and ATP were retained on the column under these conditions. The eluate was concentrated to dryness by flash evaporation at 50°C, and the residue was suspended in 5 ml 80% ethanol, filtered to remove undissolved salts, and again concentrated. The final residue was dissolved in a small volume of water. As an alternative, preparative paper chromatography was used for the isolation of labeled 5-methylthioribose-1-phosphate. The quantitation of the labeled product was based on the specific radioactivity of the labeled 5-methylthioribose used as the starting material. Paper chromatography, UV scanning, and appropriate spray reagents showed that all products described here were at least 98% pure.

Organism, enzyme extract, and incubation mixtures. Enterobacter aerogenes (ATCC 8724), a wild type strain, was used for the preparation of cell-free extracts (12). The cells were suspended in 3 vol (ml/wet wt) of 0.05 M potassium phosphate buffer, pH 7.3, containing 5 mM dithiothreitol, and passed through a French pressure cell at 20,000 p.s.i. Cellular debris was removed by centrifugation at 30,000 x g for 45 min., and the resulting supernatant fluid was used as cell-free extract.

For incubation, either 0.15 mM 5-methylthio[U-^{14}C]ribose (1.3×10^6 cpm/µmol) or 0.15 mM 5-[methyl-^{14}C]methylthioribose (2.0×10^6 cpm/µmol) was added to a reaction mixture which contained 0.05 M imidazole/HCl (pH 7.3), 10.0 mM ATP, 20.0 mM $MgSO_4$, 10.0 mM dithiothreitol, 20.0 mM NaF, 0.5 to 1.0 mg of enzyme protein in a total volume of 100 µl. After incubation for 1 h at 37°C, the reaction was terminated by the addition of 2 vol of ethanol, and the precipitated proteins and salts were removed by centrifugation. The reaction mixtures were analyzed by paper chromatography using 50 to 100 µl of the supernatant fluid.

Paper chromatography. For analytical purposes, Whatman No. 1 sheets and two solvent systems were used (Table I). For detecting radioactive spots, a radiochromatogram scanner was used. Since the area under the peaks of the graphic presentation is not always a reliable expression of the radioactivity present, the spots were cut out, and the radioactivity was determined by scintillation counting. For reference, a sample of the substrate was treated in the same way. Control experiments showed that in our system the efficiency of counting of excised paper spots was 83 ± 2% as compared with counting of identical quantities of radioactive material in solution. With distribution of the radioactive material over several spots, the losses were compounded.

For descending preparative paper chromatography, Whatman 3 mm sheets were used with solvent system A; the desired material was eluted with H_2O.

Table I

R_f Values of Methionine, 5-Methylthioribose, and Related Compounds

Compound	Solvent A	Solvent B
5-Methylthioribose	0.65	0.67
5-Methylthioribose sulfoxide	0.34	0.30
5-Methylthioribose-1-phosphate	0.22	origin
Methionine	0.48	0.40
2-Hydroxy-4-methiolbutyric acid	0.85	0.58
2-Keto-4-methiolbutyric acid	0.72	0.49
Methionine sulfoxide	0.20	0.15

The ascending solvent systems were: A, 1-butanol:acetic acid:H_2O (12:3:5); B, 2-propanol:H_2O:NH_4OH (16:3:1).

RESULTS

Paper chromatographic analysis of the products of incubation mixtures of labeled 5-methylthioribose with cell-free extracts of E. aerogenes revealed four radioactive products. The R_f values were 0.22, 0.48, 0.72, and 0.85 in solvent system A (Fig. 1). The distribution of radioactivity in the products is shown in Table II. The recovery of radioactivity in all four products, regardless of the type of labeling of the substrate, shows that the ribose part as well as the methyl group contribute to the structure of these compounds.

For identification, compounds 1 to 4 were produced in a larger quantity and isolated by preparative paper chromatography. Various tests including treatment with alkaline phosphatase, identified compound 1 as 5-methylthioribose-1-phosphate (12). Upon rechromatography of compound 2 in solvent systems A and B, the radioactive material comigrated with methionine (R_f 0.48 and 0.40). The identity of the material was confirmed by co-

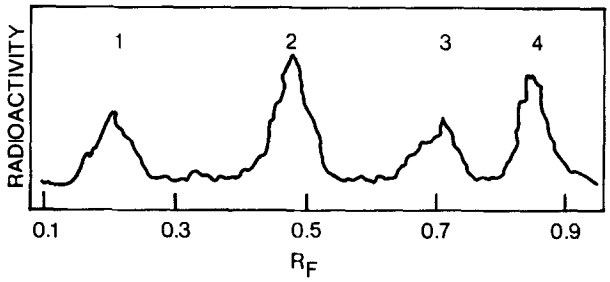

Figure 1. Radiochromatogram scan of the products formed after incubation of 5-methylthio[U-^{14}C] ribose with cell-free extract of E. aerogenes. A very similar distribution of radioactivity was observed when 5-[methyl-^{14}C]methylthioribose was used as substrate. Experimental details are described under Materials and Methods.

crystallization of the radioactive product, isolated from incubation mixtures with 5-methylthio-[U-^{14}C]ribose, with unlabeled methionine to constant specific radioactivity. After four sequential crystallizations, the values were 9.4 x 10^4, 9.3 x 10^4, 9.2 x 10^4, and 9.4 x 10^4 cpm/mmol. To corroborate the identification, a sample was treated with hydrogen peroxide; the product comigrated with methionine sulfoxide in both solvent systems (R_f 0.20 and 0.15). Incubation of another sample with L-amino acid oxidase yielded a product migrating with 2-keto-4-methiolbutyric acid in both solvent systems (R_f 0.72 and 0.49).

Compounds 3 and 4 were identified as 2-keto-4-methiolbutyric acid and 2-hydroxy-4-methiolbutyric acid by co-chromatography with reference material in solvent systems A and B (Table I). The compounds did not contain an amino group, but the methiol group was present as indicated by a positive platinum iodide test (17) and recovery of radioactivity when $^{14}CH_3$-labeled substrate was used.

Using the same chromatographic analysis, radioactive methionine was identified as a reaction product in incubation mixtures which contained either 5-[methyl-^{14}C]methylthioribose-1-phosphate or 5-methylthio[U-^{14}C]ribose-1-phosphate as the substrate.

Table II

Distribution of Radioactivity (cpm) after Incubation of Labeled 5-Methylthioribose with Cell-free Extract of Enterobacter aerogenes.

Products formed	Substrate used	
	5-Methylthio [U-^{14}C]ribose	5-[Methyl-^{14}C] Methylthioribose
5-Methylthioribose-1-phosphate	3750	7840
Methionine	6190	8255
2-keto-4-methiolbutyric acid	3680	5130
2-hydroxy-4-methiolbutyric acid	4870	6510

The amounts of deproteinized solution applied to the paper chromatograms corresponded to 20 nmol of initial substrate. The initial radioactivity was 1.3×10^6 cpm/μmol for 5-methylthio[U-^{14}C]ribose, and 2.0×10^6 cpm/μmol for 5-[methyl-^{14}C]methylthioribose. Experimental details are described under Materials and Methods.

DISCUSSION

Evidence is presented here that the ribose part of 5-methylthioribose contributes to the synthesis of the carbon chain of methionine in cell-free extracts of E. aerogenes. The metabolism of 5-methylthioribose does not merely involve the transfer of the methylthio group to a suitable four carbon acceptor. Rather, the carbohydrate moiety furnishes at least part of the four carbon chain of methionine through a series of structural modifications. The first step in the recycling appears to be conversion to 5-methylthioribose-1-phosphate (12). The presence of 2-keto-4-methiolbutyric acid and of 2-hydroxy-4-methiolbutyric acid among the reaction products probably is caused by hydrolytic and oxidative deamination of methionine although the reverse reaction sequence cannot be excluded.

The intermediate steps between 5-methylthioribose and methionine remain to be clarified, but the present experiments point toward a mechanism for the effective salvage of 5-methylthioribose. It should be recalled that the incorporation of the carbon chain of a ribose derivative into an amino acid

as suggested by the present experiments, is not unique; it has been known for some time that the five carbon chain of histidine is derived from 5-phosphoribosyl-1-pyrophosphate.

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