Conversion of 5'-S-Methyl-5-thio-D-ribose to Methionine in Klebsiella pneumoniae

STABLE ISOTOPE INCORPORATION STUDIES OF THE TERMINAL ENZYMATIC REACTIONS IN THE PATHWAY*

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Extracts of Klebsiella pneumoniae convert 5'-S-methyl-5-thio-D-ribose (methylthioribose) to methionine and formate. To probe the terminal steps of this biotransformation, [1-13C]methylthioribose has been synthesized and its metabolism examined. When supplemented with Mg2+, ATP, L-glutamine, and dioxygen, cell-free extracts of K. pneumoniae converted 50% of the [1-13C]methylthioribose to [13C]formate. The formation of [13C]formate was established by 13C and 1H NMR spectroscopy studies of the purified formate, and by 13C and 1H NMR spectroscopy and mass spectrometry studies of its p-phenylphenacyl derivative. By contrast, no incorporation of label from [1-13C]methylthioribose into the biosynthesized methionine was detected by either mass spectrometry or 13C and 1H NMR spectroscopy. The most reasonable interpretation of these results is that C-1 of methylthioribose is converted directly to formate concomitant with the conversion of carbon atoms 2-5 to methionine. The penultimate step in the conversion of methylthioribose to methionine and formate is an oxidative carbon-carbon bond cleavage reaction in which an equivalent of dioxygen is consumed. To investigate the fate of the dioxygen utilized in this reaction, the metabolism of [1-13C]methylthioribose in the presence of 16O2 was also examined. Mass spectrometry revealed the biosynthesis of substantial amounts of both [1-13C]methionine and [13C,16O]formate under these conditions. These results suggest that the oxidative transformation in the conversion of methylthioribose to methionine and formate may be catalyzed by a novel intramolecular dioxygenase. A mechanism for this dioxygenase is proposed.

Although 5'-S-methyl-5'-thioadenosine (methylthioadenosine) is the product of numerous enzymes acting on both S-adenosyl-L-methionine (AdoMet) and decarboxylated AdoMet, organisms normally contain only trace quantities of this nucleoside (1, 2). Tracer experiments have demonstrated that the methylthioribose moiety of methylthioadenosine is converted to methionine in mammals (3-7), plants (8-10), and microorganisms (11-14). This methionine salvage pathway has been most extensively characterized in the Gram-negative bacterium Klebsiella pneumoniae. The results of these studies are summarized in Scheme 1. In the first step of this conversion, a specific nucleosidase catalyzes the hydrolysis of methylthioadenosine (compound 1) to 5'-S-methyl-5-thio-D-ribose (methylthioribose, compound 2) and adenine (15-17). Phosphorylation of the alpha-oriented C-1 hydroxyl group of methylthioribose by a specific kinase produces glycosyl phosphate 3. This compound is subsequently oxidatively cleaved to alpha-keto acid 6 and formate. Dioxygen is consumed during this process. In addition, variable amounts of 3-S-methyl-3-thiopropionate (not shown) are also produced from diketone 5. The observed stoichiometry is consistent with: 1) 1 mol of O2 consumed per mol of either alpha-keto acid 6 or 3-S-methyl-3-thiopropionate produced and 2) 1 equivalent of formate generated per mol of alpha-keto acid 6 and 2 equivalents of formate generated per mol of 3-S-methyl-3-thiopropionate synthesized. Preliminary evidence suggests the possibility of a nonphosphorylated intermediate between diketone 5 and alpha-keto acid 6 and/or 3-S-methyl-3-thiopropionate (18). More recent studies suggest that the synthesis of alpha-keto acid 6 and 3-S-methyl-3-thiopropionate from diketone 5 is effected by different enzymes. In the last step of the salvage pathway, alpha-keto acid 6 is transaminated to methionine (compound 7).

To understand the metabolic conversion of methylthioadenosine to methionine in K. pneumoniae, further characterization of the reaction(s) leading from diketone 5 to alpha-keto acid 6 and formate was required. Unfortunately, studies of this transformation have been hampered by: 1) the difficulty of obtaining sufficient quantities of diketone 5, which is currently available only via a lengthy sequence of chemical and enzymatic steps; 2) the lability of certain enzymes in the pathway (including an enzyme required for the preparation of 5); and 3) the instability of diketone 5. By contrast, methylthioribose is a readily accessible and relatively stable compound; furthermore, the mechanism of its conversion to diketone 5 is well understood. Thus, in an attempt to gain further insight into the mechanism by which diketone 5 is converted into alpha-keto acid 6 and formate, we decided to study this transformation in crude cell-free extracts of K. pneumoniae. These studies employed methylthioribose as the precursor and stable isotope labeling to follow the conversion.

It seems likely that during the conversion of diketone 5 to...
formate and α-keto acid 6, C-1 of diketone 5 (C-1 of methylthioribose) is converted to formate, whereas carbon atoms 2-5 of diketone 5 yield carbon atoms 1-4 of α-keto acid 6 (19). However, Volk and Bacher (20) have recently provided evidence that the enzymatic conversion of ribulose 5-phosphate to 3,4-dihydroxybutanone-4-phosphate and formate is accompanied by a carbon chain rearrangement in which C-4 of the precursor is lost as formate and C-5 of the precursor becomes covalently bonded to C-3. Because the reactions between diketone 5 and α-keto acid 6 and formate (Scheme 1) were uncertain, we wished to provide direct evidence for the fate of the carbon atoms of methylthioribose during its enzymatic conversion to methionine and formate. Accordingly, [1-13C]methylthioribose was synthesized and its metabolism by cell-free extracts of K. pneumoniae studied.

As indicated in Scheme 1, conversion of diketone 5 to α-keto acid 6 and formate is accompanied by the consumption of 1 mol of dioxygen/mol of diketone cleaved (19). The mechanism of this unusual oxidative carbon-carbon bond cleavage was unknown. We reasoned that valuable information on the mechanism of this reaction would be obtained if the fate of the dioxygen consumed during this transformation could be established. To this end, the metabolism of [1-13C]methylthioribose in the presence of 18O2 was examined. Depending on the mechanism of the 4-electron oxidation of diketone 5 to α-keto acid 6 and formate, one would expect to observe: 1) no incorporation of 18O into either methionine or formate (18O2 conversion to H218O); 2) incorporation of one atom of 18O into either methionine or formate, the other atom of 18O being converted into H218O (monooxygenase activity); or 3) incorporation of one atom of 18O into both methionine and formate (dioxygenase activity).

**EXPERIMENTAL PROCEDURES**

**RESULTS AND DISCUSSION**

C-1 of Methylthioribose Is Converted to Formate by a Cell-Free Extract of K. pneumoniae—To determine directly the metabolic fate of C-1 of methylthioribose, 98% [1-13C]methylthioribose was synthesized and subsequently incubated with a dialyzed cell-free extract of K. pneumoniae in the presence of Mg2+, ATP, L-glutamine, and oxygen. From the incubation mixture, methionine was isolated by a combination of cation...
exchange chromatography and reverse phase HPLC. Total formate was isolated by repetitive bulb-to-bulb distillation. A parallel incubation, lacking methylthioribose, was carried out in an identical fashion. The amounts of methionine and formate isolated from both incubations were determined, using the ninhydrin and formate dehydrogenase assays, respectively (Table I). Both products generated from [1-13C]methylthioribose were then examined by 1H and 13C NMR spectroscopy. In addition, an aliquot of the purified formate was derivatized to p-phenylphenacyl formate which was purified by normal phase silica HPLC. The p-phenylphenacyl formate was also examined by 1H and 13C NMR spectroscopy and both it and methionine were analyzed by direct inlet, chemical ionization mass spectrometry.

NMR and MS analysis demonstrated that 13C enrichment was present in formate, but not in methionine, biosynthesized from [1-13C]methylthioribose. That [13C]formate was indeed a product of [1-13C]methylthioribose metabolism was established by NMR in the following manner. The 1H NMR spectrum of a 0.6 mM solution of sodium formate biosynthesized from [1-13C]methylthioribose contained a singlet (δ = 8.24 ppm) and a doublet (δ = 8.24 ppm, JCH = 194.8 Hz) corresponding to the H5CCOONa+ and H4CCOONa+ species, respectively. Integration of these signals revealed the ratio of H5CCOONa+/H4CCOONa+ to be 1.0/1.0. The proton-decoupled 13C NMR spectrum of this sample contained an intense singlet (δ = 171.9 ppm) corresponding to H5CCOONa+. The area of this signal was ~40 times greater than the signal arising from a 0.6 mM solution of natural abundance sodium formate (δ = 171.9 ppm). Use of the APT pulse sequence demonstrated that a single hydrogen atom was bonded to the carbon atom giving rise to the singlet at δ = 171.9 ppm. Upon acidification of the sample, the expected upfield shifts were observed for the proton resonances of H4CCOOH (singlet, δ = 8.03 ppm) and H5CCOOH (doublet, δ = 8.03 ppm, JCH = 218.6 Hz), as well as for the carbon resonance of H5CCOOH (singlet, δ = 166.6 ppm).

p-Phenylphenacyl formate, derived from the formate biosynthesized from [1-13C]methylthioribose, was also examined by NMR. The 1H and 13C NMR spectra of this sample were superimposable upon those of authentic natural abundance p-phenylphenacyl formate except for: 1) the presence of an additional doublet in the 1H NMR spectrum (δ = 8.25 ppm, JCH = 218.6 Hz) corresponding to H5CCOCH2CH2CO13C6H5 (cf. H5CCOCH2CH2CO12C6H5, singlet, δ = 8.25 ppm), and 2) the pronounced enhancement (36-fold) of the singlet at δ = 160.1 ppm which corresponds to H5CCOCH2CO13C6H5.

The results of chemical ionization MS analysis further demonstrated that [13C]formate was produced from [1-13C]methylthioribose (Table II). The mass spectrum of natural abundance p-phenylphenacyl formate exhibited the base peak at m/e 241 (molecular ion + H+ (MH+)), as well as major peaks of interest at m/e 87 (HCOOCH2CO13C6H5), 269 (M + C6H5CO13C6H5), and 281 (M + C6H5CO13C6H5). The corresponding +1 and +2 isotope satellite peaks were also observed for each of these signals and their signal intensities agreed well with theory. By contrast, the mass spectrum obtained for p-phenylphenacyl formate derived from the biosynthesized formate exhibited substantial enhancement of the +1 signals at m/e 242 (MH+ for H5CCOCH2CO13C6H5), 88, 270, and 282 due to 13C enrichment of the biosynthesized formate. Analysis of the MS data revealed the ratio of [13C]formate/[12C]formate to be 0.8/1.0, in accord with the average values of 1.1/1.0 and 0.5/1 obtained from H and 13C NMR spectroscopy. Thus, through a combination of physical techniques, it was established that C-1 of methylthioribose is converted to formate during its metabolism by cell-free extracts of K. pneumoniae.

By contrast, chemical ionization mass spectrometry and NMR spectroscopy established the absence of 13C enrichment in the methionine biosynthesized from [1-13C]methylthioribose. The mass spectrum of natural abundance methionine exhibited major peaks of interest at m/e 190 (M + C12H26N5O8), 178 (M + C12H26N5O7), 150 (MH+), 133 (MH+ - NH3) and 104 (MH+ - CO2H); the +1 and +2 isotope satellite peaks were also observed for these signals, and their intensities agreed well with theory. The chemical ionization mass spectrum of methionine derived from [1-13C]methylthioribose was identical in all respects to the spectrum obtained for natural abundance methionine. Thus, no enhancement was observed for any of the 13C signals of the biosynthesized methionine nor were any 13C-13C couplings detected by 13C NMR spectroscopy. The NMR spectrum of this sample contained an intense singlet (δ = 8.24 ppm) and a doublet (δ = 8.24 ppm, JCH = 194.8 Hz) corresponding to the H5CCOONa+ and H4CCOONa+ species, respectively. Integration of these signals revealed the ratio of H5CCOONa+/H4CCOONa+ to be 1.0/1.0. The proton-decoupled 13C NMR spectrum of this sample contained an intense singlet (δ = 171.9 ppm) corresponding to H5CCOONa+

As seen from the data in Table I, 50% of the 13C label that was added as [1-13C]methylthioribose can be accounted for an isolated [13C]formate. Thus, a major, if not the exclusive, metabolic product from C-1 of methylthioribose is formate. Substantial amounts of methionine, lacking 13C enrichment, were also biosynthesized from [1-13C]methylthioribose during this experiment (Table I; cf. Table III). The most reasonable interpretation of these results is that C-1 of methylthioribose is converted directly to formate concomitant with the formation of methionine from carbon atoms 2-5 of methylthioribose. This interpretation is consistent with the results of studies performed with purified diketone 5 and partially purified enzymes from K. pneumoniae, in which 1 equivalent of formate/mol of α-keto acid 6 (the direct precursor of methionine) was produced (19). Our data do not rule out the possibility that some other 1-carbon species, which is rapidly and quantitatively converted to formate, is produced from C-1 of methylthioribose during methionine biosynthesis. However, formaldehyde was not detected as a product of the conversion of diketone 5 to α-keto acid 6 and formate in a partially purified system (19). Taken together, these results suggest that C-1 of diketone 5 is converted directly to formate concomitant with the formation of α-keto acid 6 from carbon atoms 2-5.

The data in Table I also demonstrate the biosynthesis of substantial amounts of natural abundance formate from [1-13C]methylthioribose, which presumably results from alteration of the metabolic pathway by cell-free extracts of K. pneumoniae.

1 The observed increases in the signals corresponding to the +1 and +2 isotope satellite peaks for the 13C, 15O, and 18O-labeled species agreed well with the theoretical increases expected in all cases.

4 The concentration of sodium formate in this sample was determined with formate dehydrogenase.

5 We estimate that ~14 μmol of methionine was biosynthesized from [1-13C]methylthioribose in the experiment presented in Table I. This estimate is based on the results of previous studies in which we established that the recovery of methionine, when purified as described for the experiment presented in Table I, is ~40% (34). This estimate agrees well with the value of 15.5 μmol of methionine biosynthesized in the similar experiment presented in Table III. Note that the same amount of [13C]formate was produced in both experiments.
Methylthioribose Metabolism in K. pneumoniae

Methylthioribose metabolism processing of [1-13C]methylthioribose leading to 3-S-methyl-3-thiopropanoate (19). Furthermore, the amount of [13C]formate produced from [1-13C]methylthioribose was consistently greater than the amount of methionine produced (Tables I and III).6 These data suggest that the conversion of methylthioribose to 3-S-methyl-3-thiopropanoate results in the formation of formate from C-1 and apparently also from C-2, whereas 3-S-methyl-3-thiopropanoate originates from carbon atoms 3-5 of methylthioribose. This suggestion is consistent with the stoichiometry observed in a partially purified system of 2 mol of formate generated per mol of 3-S-methyl-3-thiopropanoate produced from diketone 5 (19).

Conversion of [1-13C]Methylthioribose to [13C,18O]Formate and [13C,18O]Methionine by Cell-free Extracts of K. pneumoniae in the Presence of 18O2. In the terminal steps of the methionine salvage pathway, diketone 5 undergoes a 4-electron, oxidative carbon-carbon bond cleavage, coincident with the consumption of 1 mol of O2. This process ultimately yields two carboxylic acids, namely formate and α-keto acid 6 (Scheme 1; Ref. 19). The mechanism of this unusual oxidative transformation is of interest. Accordingly, an experiment was performed in which [1-13C]methylthioribose was incubated with an undialyzed cell-free extract of K. pneumoniae in the presence of Mg2+, ATP, L-glutamine, and 98.3% 18O-labeled dioxygen. From the incubation mixture, methionine and formate were isolated. The purification scheme employed was specifically designed to minimize the exposure of methionine and formate to acidic conditions, to avoid the loss of enzymatically incorporated 18O in the resulting carboxylic acids. However, the use of acidic conditions during purification could not be completely avoided and therefore loss of 18O from the biosynthesized methionine and formate was anticipated. A parallel incubation, lacking methylthioribose, was carried out in an identical fashion. The amounts of methionine and formate produced in both incubations were determined, respectively, by radiochemical dilution and the formate dehydrogenase assay (Table III). A portion of the isolated formate was then derivatized to p-phenylphenacyl formate which was purified by normal phase silica HPLC. This sample, as well as the isolated methionine sample, were then analyzed by chemical ionization mass spectrometry.

Mass spectrometry established that metabolism of [1-13C]methylthioribose in the presence of 18O2 yields significant quantities of [13C,18O]formate and [13C,18O]methionine. That [18O]methionine is isolated from the incubation containing [1-13C]methylthioribose and 18O2 is demonstrated by the data in Table IV. For example, whereas the relative ratio of the signal intensities for the peaks at m/e 152 versus 150 ([MH + 2]+ and MH+, respectively) and 155 versus 153 ([MH - NH3]+ and [MH - NH2]+, respectively) were both 0.06/1.0 for natural abundance methionine, these ratios were increased to 1.4/1.0 and 1.2/1.0 in the methionine sample isolated from the incorporation experiment. By contrast, the ratio of the signal intensities for the peaks at m/e 106 and 104 ([MH - CO2H2]+ + 2) and (MH - CO2H2)+, respectively) were essentially identical for both the natural abundance and biosynthetic methionine samples. Such data demonstrated the incorporation of one atom of 18O from 18O2 into a portion of the methionine isolated from the incubation containing [1-13C]methylthioribose.

Based on the MS data, the ratio of [18O]/[16O]methionine isolated from the incorporation experiment was 1.3/1. Thus, 56% of the isolated methionine contained one atom of 18O from 18O2 in its carboxyl group. No evidence for the formation of [18O]methionine was found. We observed the attributed lower than stoichiometric incorporation of 18O into the isolated methionine primarily to chemically induced washout of the biosynthesized [18O]methionine during its purification. This washout most likely occurred as a result of the strongly acidic conditions encountered during the cation exchange chromatography step. Strongly acidic conditions are well known to catalyze washout of 18O-labeled carboxylic acids, including amino acids (21, 22). Based on these results, it seems most likely that the conversion of methylthioribose to methionine results in the incorporation of one atom of oxygen from dioxygen into the carboxylate group of the resultant methionine.

Similarly, the results in Table IV established the incorporation of one atom of 18O into a portion of the [13C]formate biosynthesized from [1-13C]methylthioribose in the presence of 18O2. For example, whereas the relative ratio of the signal intensities for the peaks at m/e 244 versus 241 ((MH + 3)+ and MH+, respectively) and 90 versus 87 ([HCO2.CH:CO + 3]+ and HCO2.CH:CO+, respectively) were both 0.00/1 for natural abundance p-phenylphenacyl formate, these ratios were increased to 0.05/1 in the p-phenylphenacyl formate sample derived from the formate isolated from the incorporation experiment. Such data demonstrated the biosynthesis of [13C,18O]formate from [1-13C]methylthioribose and 18O2. No evidence for the formation of [13C,18O]formate was found.

Further confirmation of the biosynthesis of [13C,18O]formate was obtained by 13C NMR spectroscopy. The 13C NMR spectrum of the biosynthetic p-phenylphenacyl formate sample contained the expected enriched signal at δ = 160.07 ppm corresponding to the H3C:CO2.CH:COC12H5 species. In addition, a shoulder at δ = 160.04 ppm (0.03 ppm upfield) was observed which corresponds to the H3C:CO2.H:O2.CH:COC12H5 species. The 13C chemical shift of the carbonyl carbon of esters is known to shift upfield -0.025 ppm upon isotopic substitution of one of the ester oxygens with 18O (23). Hence, no evidence for the formation of [13C,18O]formate was found.

Based on the MS data, the ratio of [13C,18O]/[1-13C,16O] formate isolated from the incorporation experiment was 0.2/1. Thus, 19% of the isolated [13C,18O]formate contained one atom of 18O from 18O2. As before, we attribute the less than stoichiometric incorporation of 18O into the isolated [13C,18O]formate to chemically induced washout of the biosynthesized [13C,18O]formate during its purification. Most likely, the bulb-to-bulb distillation step (pH 1.3, ~0.7 M HClO4) caused the majority of this washout. To confirm this notion, an aliquot of the formate recovered in the second distillate was derivatized to the p-phenylphenacyl ester and analyzed by MS. The results of this experiment (not shown) demonstrated the absence of 18O in the [13C]formate present in the second distillate. The additional loss of 18O is due to further chemical washout caused by the increased exposure to acidic conditions. Other experiments by Corina (24) have demonstrated 76% washout of 18O from [13C]formate upon incubation in 1 N aqueous hydrochloric acid for 45 min at 25 °C. Based on these data, it seems most likely that the conversion of C-1 of methylthioribose into formate results in the incorporation of one atom of oxygen from dioxygen into the resultant formate.

Quantitative analysis (Table III) demonstrated the biosynthesis, from 50 μmol of [1-13C]methylthioribose, of 15.5 μmol of methionine, 23.4 μmol of [13C]formate, and 15.1 μmol of natural abundance formate. As discussed earlier, we interpret these results to mean that, under our conditions, [1-13C]methylthioribose is converted into [13C]formate and methionine, and that alternate metabolism of [1-13C]methylthioribose to 3-S-methyl-3-thiopropanoate occurs; the latter process yields both 13C and natural abundance formate (cf. Ref. 19).
The most reasonable interpretation of these results is that the conversion of diketone 5 to α-keto acid 6 and formate results in the incorporation of one atom of oxygen from dioxygen into the carboxylate group of both products.

Based on these arguments, we suggest that the oxidative carbon-carbon bond cleavage in the conversion of methylthioribose into methionine and formate is effected in a single reaction catalyzed by a novel intramolecular dioxygenase. A speculative mechanism, consistent with the presently available data, for the dioxygenase-mediated conversion of diketone 5 into α-keto acid 6 and formate is presented in Scheme 2. According to this mechanism, an enzyme generated, phosphorylated enediol 8 serves as the nucleophile for attack on dioxygen. By analogy, a similar carbanion mechanism has been proposed for substrate oxygenation by ribulose-1,5-bisphosphate oxygenase (25). Dioxygen may be enzymatically activated for nucleophilic attack by, for example, metal catalysis. The nucleophilic oxygen atom of the C-1 peroxide group of the resulting intermediate (9) then attacks the electrophilic carbonyl carbon at C-2, yielding intermediate 10. Decomposition of dioxetane 10 into α-keto acid 6 and formate is then initiated by hydrolysis of the C-1 phosphate ester. Alternatively, decomposition of dioxetane 10 might be initiated by deprotonation of the C-2 hydroxyl group, in which case α-keto acid 6 and formyl phosphate would be produced (not shown). Due to its hydrolytic instability (26), any formyl phosphate formed in our incubations would be converted to formate during work-up. Clearly, other variations of the mechanism shown in Scheme 2 exist. Notable among these is the possibility that oxygenation of the enediol-phosphate 8 occurs at C-2; C-2 carbanion formation being initiated by hydrolysis of the C-1 phosphate ester; in this case, the intermediate dioxetane 10 would not be phosphorylated. It should be noted.
that dioxetanes have frequently been suggested as intermediates in dioxygenase catalyzed reactions, including the reactions catalyzed by intra- and extra-diol pyrrolases (27) and by bioluminescence-producing enzymes, e.g. luciferase (28).

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corresponding substrate was complete by 20 min. After 20 min, in the solution of sodium methionine (0.06 M), no methionine was detected in the medium. Following the addition of 30 min at 0°C, the reaction was brought to room temperature and stirred. The reaction was carried out for 20 min. It was found that the reaction was complete. At that time, water (10 ml), then chloroform (10 ml), were added to the mixture, separated, and the chloroform was evaporated to dryness. The residue was dissolved in a small volume of toluene, applied to a column of silica gel (12 g) and eluted with solvent C. Fractions containing the pure major product were pooled and evaporated to dryness. The residue was dissolved in 1:10 methanol-ethyl acetate and purified by paper chromatography on Whatman No. 1 filter paper with solvent (7). The major product was obtained in crystalline form. Yield 145 mg (17%).


glycerol-negative C. albicans (strain M15) was grown on yeast extract, malt extract and glucose plate at 30°C for 24 h. Following the addition of 0.6 ml of potassium phosphate buffer (pH 7.4) to the culture, the cells were harvested by centrifugation, washed with water (3), and suspended in 1 ml of 0.1 M Tris-HCl buffer (pH 7.4). The cell suspensions were then incubated at 37°C for 30 min. The mixture was centrifuged at 3000 g for 10 min and the supernatant fluid was used as the enzyme source.


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Table I. Quantities of Formate and Methionine Isolated Following Incubation of [1,3-13C]Methylthioribose with K. pneumoniae Cell-Free Extract.

| Precursor Added | Product | Amount of product isolated (pmol) | Amount of isolated precursor biosynthesized from [1,3-13C]methylthioribose (pmol) |
|-----------------|---------|----------------------------------|--------------------------------------------------------------------------------|
| None            | [12C]Formate | 6.5 ± 0.6                         | ---                                                                    |
| [12C]Formate    | g.l.a.   | ---                              | ---                                                                    |
| [12C]Methionine | 1.2 ± 0.5 | ---                              | ---                                                                    |
| [12C]Methionine | 0.6 ± 0.4 | ---                              | ---                                                                    |
| [13C]Methionine (500 pmol) | [12C]Formate | 31.5 ± 0.43                     | 25.0                                                                  |
|                  | [12C]Formate | 16.7 ± 0.3                       | 10.4                                                                  |
|                  | [12C]Methionine | 6.0 ± 0.5                      | 5.7                                                                  |
|                  | [12C]Methionine | 0.6 ± 0.4                      | 0                                                                    |

a) Values given represent experimental values multiplied by 2 to compensate for the lower amount of extract used in this experiment.
b) Total formate isolated after exhaustive distillation determined with formate dehydrogenase.
c) Methionine isolated determined by ninhydrin assay coupled with purity assessment by 5-methyltetrahydropteridine and HPLC.
d) Based on [12C]/[13C]formate ratio of 0.88/1.0 obtained by MS analysis.

Table II. Isotopic Content of the Formate and Methionine Isolated Following Incubation of [1,3-13C]methylthioribose with K. pneumoniae Cell-Free Extract.

| Enzyme | N-Relative Intensity | 24h | 28h | 32h | 36h | 40h | 44h |
|--------|----------------------|-----|-----|-----|-----|-----|-----|
| Natural Abundance-Theoretical | 24h | 28h | 32h | 36h | 40h | 44h |
| Natural Abundance-Experimental | 24h | 28h | 32h | 36h | 40h | 44h |
| Biosynthetic | 13C | 17C | 13C | 17C | 13C | 17C |
| Methionine | 6.8 | 6.4 | 5.6 | 4.6 | 4.6 | 4.6 |
| Natural Abundance-Theoretical | 24h | 28h | 32h | 36h | 40h | 44h |
| Natural Abundance-Experimental | 24h | 28h | 32h | 36h | 40h | 44h |
| Biosynthetic | 13C | 17C | 13C | 17C | 13C | 17C |

a) For p-lactamase formed. b) For methionine: 13C-12C, 13C-12C, 13C-12C, 13C-12C, 13C-12C. c) 13C-12C (13C-12C).

The % relative intensities within a given group of lanes were calculated by assigning a value of 100% to the absolute abundance for the parent ion (241.07/161.13/141.04) of the natural abundance mixture.

Table III. Quantities of Formate and Methionine Biosynthesized from [1,3-13C]methylthioribose in the Presence of [13C]formate.

| Precursor Added | Product | Amount of product present at end of incubation (pmol) | Amount of product biosynthesized from [1,3-13C]methylthioribose (pmol) |
|-----------------|---------|------------------------------------------------------|----------------------------------------------------------------------|
| None            | [12C]Formate | 94.2 ± 0.6                                   | ---                                                                  |
| [12C]Formate    | g.l.a.   | ---                                              | ---                                                                  |
| [12C]Methionine | 8.6 ± 0.4 | ---                                              | ---                                                                  |
| [12C]Methionine | 0.6 ± 0.4 | ---                                              | ---                                                                  |
| [13C]Methionine (100 pmol) | [12C]Formate | 109.6 ± 0.4                        | 15.1                                                                  |
|                  | [12C]Formate | 22.4 ± 0.4                        | 4.4                                                                  |
|                  | [12C]Methionine | 24.5 ± 0.5                    | 15.5                                                                  |
|                  | [12C]Methionine | 0.6 ± 0.4                    | 0                                                                    |

a) Values given represent experimental values multiplied by 2 to compensate for the lower amount of extract used in this experiment.
b) Total formate isolated after exhaustive distillation determined with formate dehydrogenase.
c) Total methionine present at end of incubation determined by isotope dilution.
d) Based on [12C]/[13C]formate ratio of 0.88/1.0 determined by MS analysis. This value agrees with those obtained by GC and GC-MS.
### Methylthioribose Metabolism in K. pneumoniae

| Compound                        | % Relative Intensity at mass |
|---------------------------------|-----------------------------|
| **Methylthioribose**            |                             |
| Natural Abundance—Theoretical   | 100\(^\text{a}\)            |
| Natural Abundance—Experimental  | 100\(^\text{b}\)            |
| Biosynthesized                  | 100\(^\text{c}\)            |
| **g-Phenyl-hexonol Formate**    |                             |
| Natural Abundance—Theoretical   | 100\(^\text{a}\)            |
| Natural Abundance—Experimental  | 100\(^\text{b}\)            |
| Biosynthesized                  | 100\(^\text{c}\)            |

\(^\text{a}\) For methylthioribose: 150 [Mm\(^+\)], 151 [C\(_5\)H\(_9\)NO\(_3\)A], 133 [Mm\(^{-}\)], and 104 [M\(_2\)O\(_2\)P\(_2\)\(^{32}\)]\(^+\); for g-phenyl-hexonol: 241 [M\(_2\)N\(_2\)P\(_2\)]\(^+\) and 277 [M\(_2\)O\(_2\)P\(_2\)C\(_6\)H\(_5\)]\(^+\).

\(^\text{b}\) The % relative intensities within a given group of ions were calculated by assigning a value of 100 to the absolute abundance for the ion at 1246.97. 150, 153, 154 due to the natural abundance species.

\(^\text{c}\) Also the two cases were shown up to be uncertain due to the very minor absolute abundance of the two differing ions compared to the relatively low absolute abundance of the ions in question.
Conversion of 5-S-methyl-5-thio-D-ribose to methionine in Klebsiella pneumoniae. Stable isotope incorporation studies of the terminal enzymatic reactions in the pathway.

R W Myers and R H Abeles

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