

Biosynthesis of Corrinooids and Porphyrinooids. XI. Source of Oxaloacetic Acid for Uroporphyrinogen III Biosynthesis in *Arthrobacter hyalinus*

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The source of oxaloacetic acid, required for the synthesis of porphyrins in *Arthrobacter hyalinus*, was examined by means of a feeding experiment with $[1,3-^{13}\text{C}_2]$ glycerol, which is transformed to pyruvic acid. A half of the carbon dioxide liberated from pyruvic acid in the formation of acetyl CoA was utilized for carboxylation of pyruvic acid to generate oxaloacetic acid.

Key words *Arthrobacter hyalinus*; tricarboxylic acid cycle; uroporphyrin III; $[1,3-^{13}\text{C}_2]$ glycerol

Arthrobacter hyalinus synthesizes porphyrins,¹ and we have already examined its biopathways using several ^{13}C -labeled compounds.^{2,3)} ^{13}C -Labeled isopropanol or sodium acetate was transformed into ^{13}C -labeled acetyl CoA, which condensed with oxaloacetic acid to form ^{13}C -labeled citric acid, which ultimately afforded ^{13}C -labeled porphyrins. To identify the source of the oxaloacetic acid, we have conducted a feeding experiment with $[1,3-^{13}\text{C}_2]$ glycerol in *A. hyalinus*.

Results and Discussion

The ^{13}C -NMR spectrum (Fig. 1) of the octamethyl ester derived from ^{13}C -labeled uroporphyrinogen III biosynthesized from $[1,3-^{13}\text{C}_2]$ glycerol in *A. hyalinus* showed ^{13}C -enriched signals at 21.9 ppm (β -methylene carbons of propyl side-chains), 32.7 ppm (α -methylene carbons of acetyl side-chains), 37.1 ppm (α -methylene carbons of

propyl side-chains), 133.1 ppm (C-2, 7, 12, 18), 141.0 ppm (C-3, 8, 13, 17) and 143.8 ppm (C-1, 6, 11, 19). This ^{13}C -enrichment pattern showed that $[1,3-^{13}\text{C}_2]$ glycerol had been converted into ^{13}C -labeled porphyrin through the same route as that we reported previously for incorporation of $[2-^{13}\text{C}]$ sodium acetate²⁾ (Figs. 1, 2). However, additional ^{13}C -enriched signals due to C-5, 10, 15 and 20 (Figs. 1, 2, Table 1) were observed. It was considered that $[^{13}\text{C}]$ carbon dioxide, liberated by decarboxylation of $[1,3-^{13}\text{C}_2]$ pyruvic acid that was derived from $[1,3-^{13}\text{C}_2]$ glycerol, condensed with pyruvic acid to produce $[4-^{13}\text{C}]$ oxaloacetic acid. This condensed with acetyl CoA, to give $[5-^{13}\text{C}]\delta$ -aminolevulinic acid via $[1-^{13}\text{C}]\alpha$ -ketoglutaric acid and $[1-^{13}\text{C}]\text{glutamic acid}$. Consequently, C-5, 10, 15 and 20 of uroporphyrin III octamethyl ester would also be ^{13}C -labeled.

Further, carbon dioxide liberated from pyruvic acid

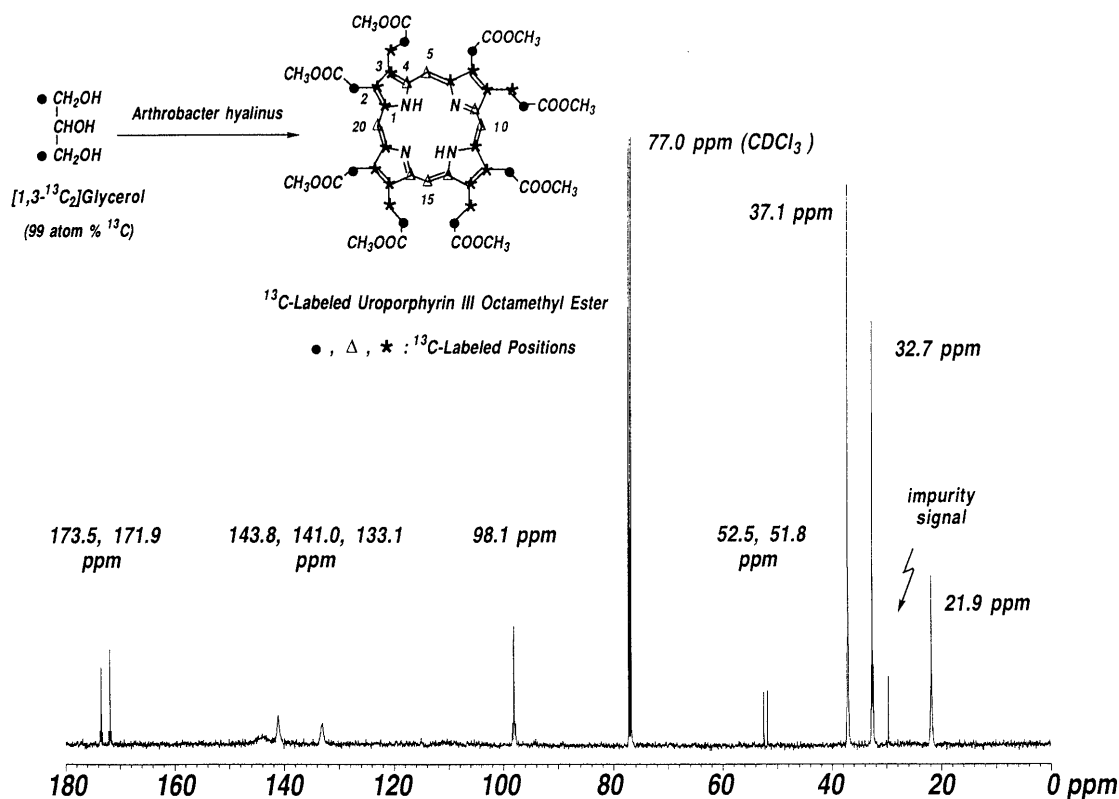


Fig. 1. Structure of Uroporphyrin III Octamethyl Ester and ^{13}C -NMR Spectrum of ^{13}C -Labeled Uroporphyrin III Octamethyl Ester Derived from $[1,3-^{13}\text{C}_2]$ glycerol

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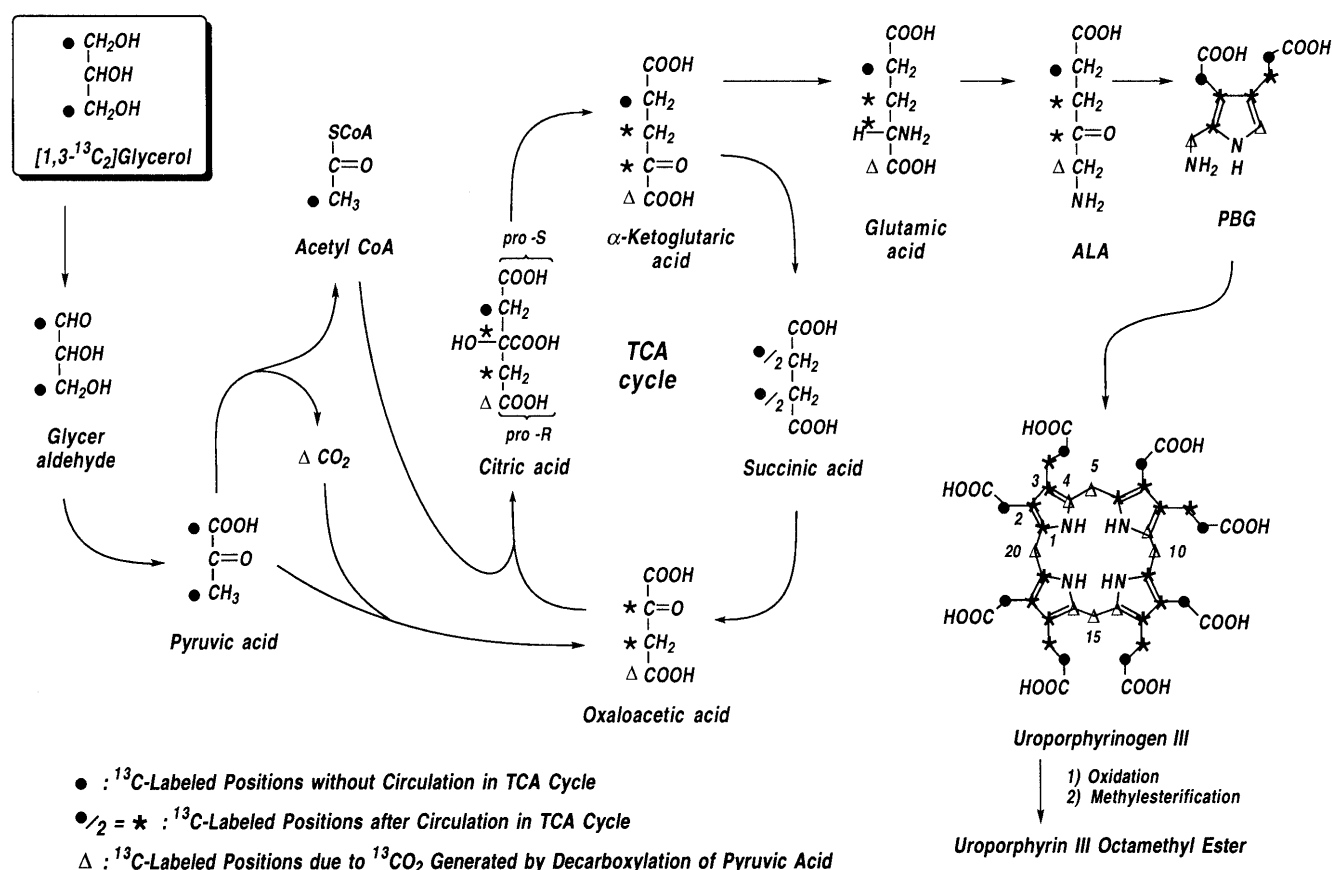


Fig. 2. Metabolic Pathways Leading from $[1,3-^{13}\text{C}_2]$ Glycerol to ^{13}C -Labeled Uroporphyrinogen III in *Arthrobacter hyalinus*

Table 1. ^{13}C -Enrichment Ratios for Carbon Atoms in ^{13}C -Labeled Uroporphyrin III Octamethyl Ester Derived from $[1,3-^{13}\text{C}_2]$ Glycerol

Positions of carbon in ^{13}C -labeled uroporphyrin III octamethyl ester	Chemical shift (coupling pattern)	Ratio of ^{13}C -enrichment
β-Methylene carbons of Ps	21.9 ppm (s)	5.3
α-Methylene carbons of As	32.7 ppm (s)	11.2
α-Methylene carbons of Ps	37.1 ppm (s)	11.3
Methyl ester carbons of Ps	51.8 ppm (s)	1.0
Methyl ester carbons of As	52.5 ppm (s)	1.0
C-5, 10, 15, 20	98.1 ppm (s)	5.9
C-2, 7, 12, 18	133.1 ppm (brs)	N.C.
C-3, 8, 13, 17	141.0 ppm (brs)	N.C.
C-1, 4, 6, 9, 11, 14, 16, 19	143.8 ppm (br)	N.C.
Carbonyl carbons of As	171.9 ppm (s)	1.6
Carbonyl carbons of Ps	173.5 ppm (s)	1.4

The "A" indicates the acetyl side-chains and the "P" indicates the propyl side-chains in ^{13}C -labeled uroporphyrin III octamethyl ester. The signals of methyl ester carbons of As and Ps are given for reference. The signals of carbon in ^{13}C -labeled uroporphyrin III octamethyl ester were compared with natural abundance in uroporphyrin III octamethyl ester to obtain the enrichment ratio. N.C. = not calculable.

was immediately used for carboxylation of pyruvic acid. Based on the ratio of ^{13}C -enrichment at C-5, 10, 15 and 20 to ^{13}C -enrichment at the α-methylene carbons of the acetyl and propyl moieties (Table 1), approximately 50% of $[^{13}\text{C}]$ carbon dioxide liberated from $[1,3-^{13}\text{C}_2]$ pyruvic acid was utilized in this way.

Experimental

Materials and Instrument $[1,3-^{13}\text{C}_2]$ Glycerol (99 atom% ^{13}C) was supplied by Icon. $^{13}\text{C}\{-^1\text{H}\}$ NMR spectra were recorded on a Varian Unity INOVA 500 (125 MHz) spectrometer with a nanoprobe in CDCl_3 solution referenced to the solvent peak. The spectral width was 25000 Hz with 65536 K data points, which corresponds to a resolution of 0.76 Hz per point. The determined 10° pulse width was 2.2 μs, the acquisition time was 1.311 s, the pulse delay time was 0.689 s and the number of scans was 10000.

Feeding of $[1,3-^{13}\text{C}_2]$ Glycerol to *A. hyalinus* $[1,3-^{13}\text{C}_2]$ Glycerol (250 mg × 4) was added to the fermentation culture medium (pH 7.0, 200 ml × 4), which consisted of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (5.0 g), CaCO_3 (5.0 g), NH_4NO_3 (3.0 g), peptone (3.0 g), $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ (1.5 g), L-cystine (0.6 g), yeast extract (1.0 g), KH_2PO_4 (0.4 g), $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (10 mg), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (50 μg), MoO_3 (10 μg) and monosodium L-glutamate (1.0 g) in ion-exchanged water (1.0 l), in 500 ml Erlenmeyer flasks. The flasks were shaken at 27 °C for 10 d on a rotary incubator (200 rpm).

Isolation of ^{13}C -Labeled Uroporphyrin III Octamethyl Ester The method of isolation of ^{13}C -labeled uroporphyrin III octamethyl ester (1.2 mg), which was produced by feeding of $[1,3-^{13}\text{C}_2]$ glycerol to *A. hyalinus*, has been detailed in the preceding papers.¹⁻³⁾

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