Biosynthesis of Corrinoids and Porphyrinoids. XI. Source of Oxaloacetic Acid for Uroporphyrinogen III Biosynthesis in *Arthrobacter hyalinus*

Katsumi IIDA, Toshiko AOKI, Ryuichi UEGAKI, and Masahiro KAJIWARA*

Department of Medicinal Chemistry, Meiji College of Pharmacy, 1–22–1 Yato-cho, Tanashi-shi, Tokyo 188, Japan.
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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-$^{13}C_2$]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-$^{13}C_2$]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-$^{13}C_2$]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-$^{13}C_2$]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-$^{13}C_2$]glycerol had been converted into ^{13}C-labeled porphyrin through the same route as that we reported previously for incorporation of [2-^{13}C]sodium acetate[2] (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}C]carbon dioxide, liberated by decarboxylation of [1,3-$^{13}C_2$]pyruvic acid that was derived from [1,3-$^{13}C_2$]glycerol, condensed with pyruvic acid to produce [4-^{13}C]oxaloacetic acid. This condensed with acetyl CoA, to give [5-^{13}C]δ-aminolevulinic acid *via* [1-^{13}C]α-ketoglutaric acid and [1-^{13}C]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

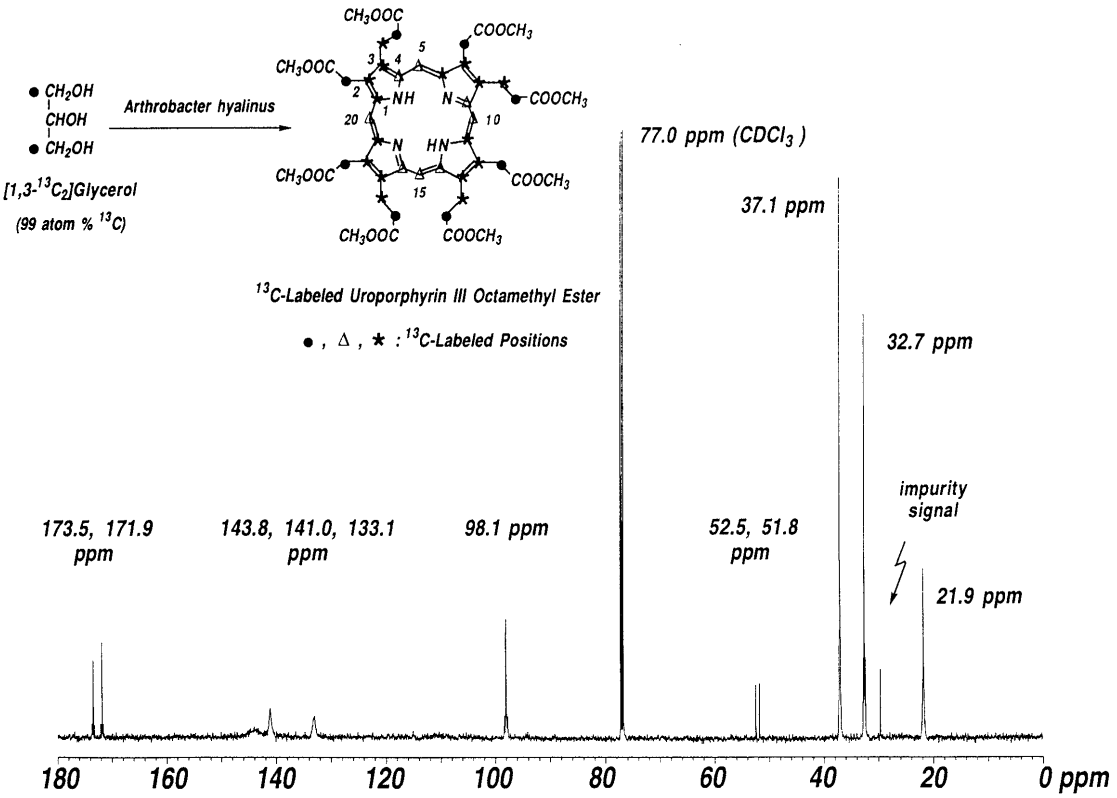

Fig. 1. Structure of Uroporphyrin III Octamethyl Ester and ^{13}C-NMR Spectrum of ^{13}C-Labeled Uroporphyrin III Octamethyl Ester Derived from [1,3-$^{13}C_2$]Glycerol

* To whom correspondence should be addressed.

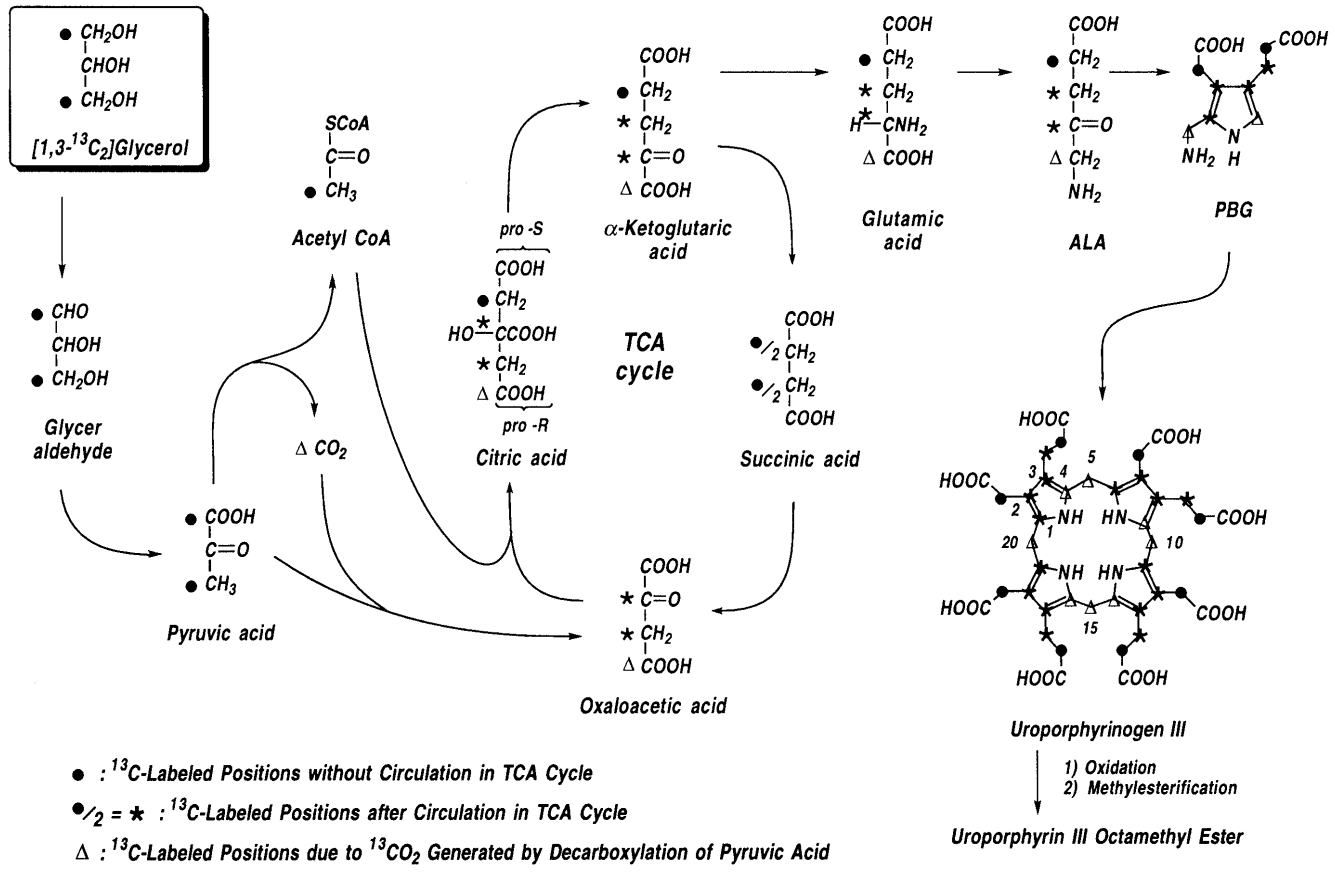

Fig. 2. Metabolic Pathways Leading from [1,3-$^{13}C_2$]Glycerol to ^{13}C-Labeled Uroporphyrinogen III in *Arthrobacter hyalinus*

Table 1. ^{13}C-Enrichment Ratios for Carbon Atoms in ^{13}C-Labeled Uroporphyrin III Octamethyl Ester Derived from [1,3-$^{13}C_2$]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 (br s)	N.C.
C-3, 8, 13, 17	141.0 ppm (br s)	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}C]carbon dioxide liberated from [1,3-$^{13}C_2$]pyruvic acid was utilized in this way.

Experimental

Materials and Instrument [1,3-$^{13}C_2$]Glycerol (99 atom% ^{13}C) was supplied by Icon. ^{13}C-{^{1}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-$^{13}C_2$]Glycerol to *A. hyalinus* [1,3-$^{13}C_2$]Glycerol (250 mg × 4) was added to the fermentation culture medium (pH 7.0, 200 ml × 4), which consisted of $MgSO_4 \cdot 7H_2O$ (5.0 g), $CaCO_3$ (5.0 g), NH_4NO_3 (3.0 g), peptone (3.0 g), $Na_2HPO_4 \cdot 12H_2O$ (1.5 g), L-cystine (0.6 g), yeast extract (1.0 g), KH_2PO_4 (0.4 g), $ZnSO_4 \cdot 7H_2O$ (10 mg), $CuSO_4 \cdot 5H_2O$ (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-$^{13}C_2$]glycerol to *A. hyalinus*, has been detailed in the preceding papers.[1-3]

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References

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