Evaluation of Biosynthetic Pathways to δ-Aminolevulinic Acid in *Propionibacterium shermanii* Based on Biosynthesis of Vitamin B_{12} from D-[1-^{13}C]Glucose

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ABSTRACT: Analysis of the ^{13}C nuclear magnetic resonance (NMR) spectrum of ^{13}C-labeled vitamin B_{12} biosynthesized from D-[1-^{13}C]glucose by *Propionibacterium shermanii* provided evidence suggesting that δ-aminolevulinic acid (ALA) incorporated in the ^{13}C-labeled vitamin B_{12} may have been synthesized via both the Shemin pathway and the C5 pathway under anaerobic conditions in the ratio of $1 < [$(ratio of ALA biosynthesis from the Shemin pathway)/(that from the C5 pathway)$] < 1.8$. The D-ribose moiety of vitamin B_{12} was labeled with ^{13}C at R-1, R-3, and R-5. The aminopropanol moiety of vitamin B_{12} was labeled on Pr-1 and Pr-2, but not Pr-3.

δ-Aminolevulinic acid (ALA)[1] (**2**), an intermediate in the biosynthesis of tetrapyrrole compounds such as vitamin B_{12} (**1**), chlorophyll, and heme, is biosynthesized via two pathways, the Shemin pathway (C4 pathway) (*1*−*5*) and the C5 pathway (*6*−*11*) (Figure 1). The Shemin pathway has been found in animals, fungi (including yeast), and the α-proteobacteria, such as photosynthetic *Rhodobacter* and *Bradyrhizobium*. In this pathway, ALA (**2**) is biosynthesized by the condensation of glycine (**3**) and succinyl-coenzyme A (CoA) (**4**) catalyzed by ALA synthase. The C5 pathway has been found in plants (including algae) and all other bacteria so far examined. This pathway is composed of the following enzyme reactions. Glutamate (glutamic acid (**5**)) is coupled with transfer ribonucleic acid (tRNA), catalyzed by glutamyl-tRNA synthase, and then reduced to afford glutamate 1-semialdehyde (GSA), catalyzed by glutamyl-tRNA reductase. Finally, transamination of GSA catalyzed by GSA aminomutase gives ALA (**2**).

Shemin and others reported that ALA (**2**) is biosynthesized via the Shemin pathway in *Propionibacterium shermanii* (*4*, *5*). They confirmed the ability of ALA synthase in *P. shermanii* to utilize glycine (**3**) and succinyl-CoA (**4**). However, Murakami et al. (*12*) showed that *Propionibacterium freudenreichii* contains GSA aminomutase, and suggested that ALA (**2**) was synthesized via the C5 pathway. We were interested in investigating the relative importance of the two biosynthetic pathways for ALA (**2**) in *P. shermanii*. Instead of glycine (**3**), succinyl-CoA (**4**), or glutamic acid (**5**), we chose D-glucose (**8**) as a stable isotope-labeled compound before the tricarboxylic acid (TCA) cycle. As shown in Figure 1, acetyl-CoA (**7**) derived from D-glucose (**8**) enters the TCA cycle and is converted into α-ketoglutaric acid (**6**) and then succinyl-CoA (**4**), which are the entry points to the C5 pathway and the Shemin pathway, respectively, leading to ALA (**2**) and then vitamin B_{12} (**1**). In a feeding experiment with D-[1-^{13}C]glucose, the ^{13}C-enrichment ratios of the carbon atoms of ^{13}C-labeled vitamin B_{12} should allow us to distinguish the biosynthetic pathways of ALA (**2**) in *P. shermanii*, as well as providing information about the biosynthetic pathways leading to other moieties (D-ribose and aminopropanol moieties) of vitamin B_{12} (**1**).

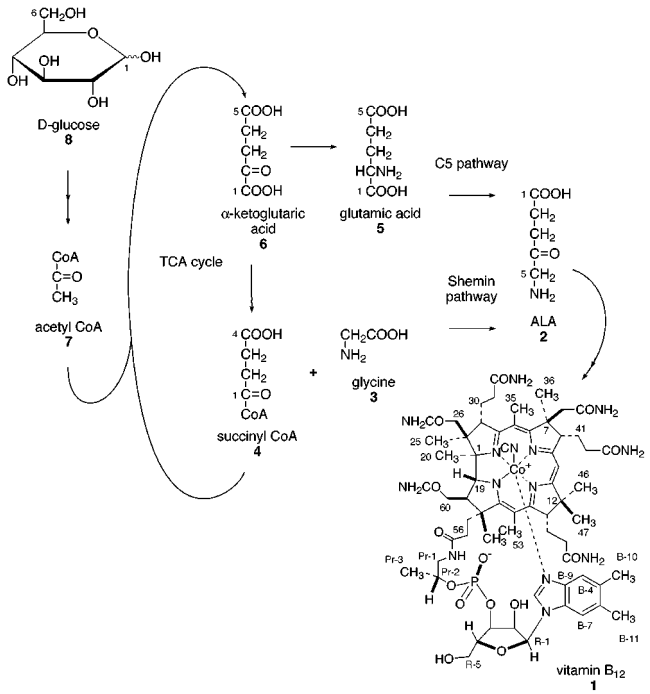

FIGURE 1: Biosynthesis of vitamin B_{12} (**1**) from D-glucose (**8**) through ALA (**2**) putatively formed via the Shemin pathway and the C5 pathway.

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[1] Abbreviations: ALA, δ-aminolevulinic acid; CoA, coenzyme A; COSY, correlation spectroscopy; DMBI, 5,6-dimethylbenzimidazole; GSA, glutamate 1-semialdehyde; NMR, nuclear magnetic resonance; TCA, tricarboxylic acid; tRNA, transfer ribonucleic acid; TSP, sodium 3-trimethylsilyl[2,2,3,3-$^{2}H_4$]propionate.

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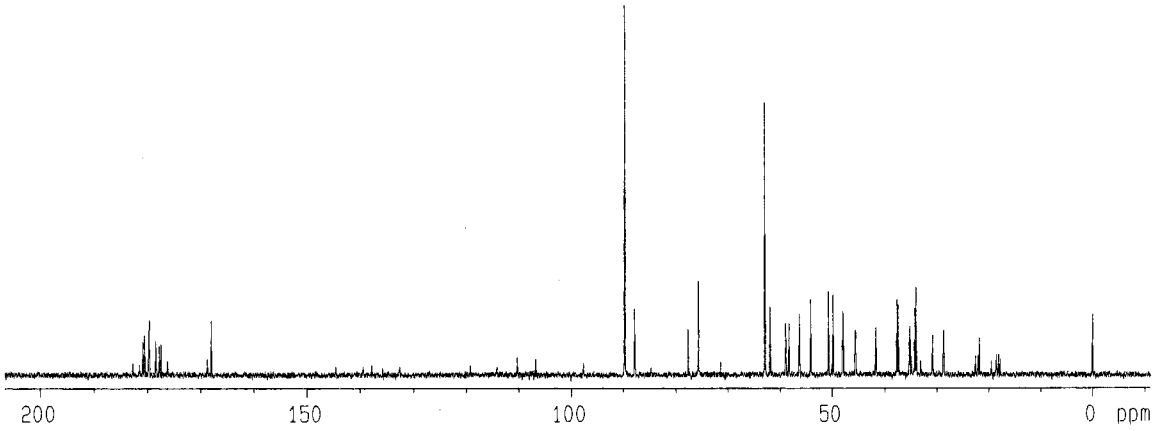

FIGURE 2: ^{13}C NMR spectrum of ^{13}C-labeled vitamin B_{12} derived from D-[1-^{13}C]glucose in *P. shermanii*.

EXPERIMENTAL PROCEDURES

Feeding of D-[1-^{13}C]Glucose to Propionibacterium shermanii. Feeding of D-[1-^{13}C]glucose and isolation of ^{13}C-labeled vitamin B_{12} were carried out by modifying the methods described in our previous papers (*13*−*15*). The cultures of *P. shermanii* ATCC 9614 were grown in seed culture medium (pH 7.0, 400 mL × 4), which consisted of D-glucose (6 g), casein (acid hydrolysates Hy-case SF, 12.5 g), NZ case (12.5 g), yeast extract (5 g), KH_2PO_4 (1.76 g), K_3PO_4 (1.76 g), $MgCl_2 \cdot 6H_2O$ (0.4 g), and $FeSO_4 \cdot 7H_2O$ (0.01 g) in ion-exchanged water (1 L), in a 1 L culture bottle at 27 °C. A sterilized solution of D-[1-^{13}C]glucose (30 g, 99 atom % ^{13}C, Cambridge Isotope Laboratories) in water (100 mL), a solution of L-methionine (100 mg) in water (5 mL), which had been filtered through a membrane filter (0.2 μm), a solution of 5,6-dimethylbenzimidazole (DMBI) (*16*, *17*) (50 mg) in 80% ethyl alcohol (2 mL), and wet cells (harvested from two seed culture media after 7 days and washed with 0.9% NaCl) were added to the fermentation culture medium (pH 7, 2 L × 2). The latter consisted of yeast extract (15 g), K_2HPO_4 (1.74 g), $NaH_2PO_4 \cdot 2H_2O$ (1.56 g), $CoCl_2 \cdot 6H_2O$ (20 mg), calcium pantothenate (2 mg), D-biotin (2 mg), and thiamine hydrochloride (2 mg) in ion-exchanged water (1 L), in a 5 L Erlenmeyer flask. The cultures of *P. shermanii* were continuously grown at 27 °C for 7 days under bubbling nitrogen gas. These media were adjusted to pH 7 with 20% Na_2CO_3, and no D-glucose was added during the fermentation.

Isolation of ^{13}C-Labeled Vitamin B_{12}. The pellet, collected by centrifugation of the culture broth for 30 min at 12300*g*, was washed with 0.9% NaCl, and this suspension was centrifuged again under the same conditions. This pellet was suspended in 80% methyl alcohol (500 mL) containing 0.1% KCN, and then this suspension was adjusted to pH 6.8 with 3 N HCl, heated under reflux for 30 min, and centrifuged for 30 min at 12300*g*. This process was repeated twice, and the combined supernatant was evaporated. The residue was dissolved in water (50 mL), and the solution was extracted with phenol−chloroform (1:1 v/v, 30 mL × 3). The combined extracts were washed with water (30 mL × 5), diluted with 10 volumes of ether, and re-extracted with water (5 mL × 3). The combined re-extracts were washed with ether (30 mL × 5) and evaporated. Chromatography on silica gel with methyl alcohol, followed by recrystallization of the product from water−acetone, gave ^{13}C-labeled vitamin B_{12} (4 mg).

^{13}C Nuclear Magnetic Resonance Spectra of Vitamin B_{12}. The ^{13}C NMR spectra were obtained for solutions (4.9 μM) of ^{13}C-labeled vitamin B_{12} and vitamin B_{12} (**1**) (Glaxo Operations U.K. Ltd.) in ^2H_2O. All spectra were recorded on a Jeol LA-500 (125 MHz) spectrometer with a solution of sodium 3-trimethylsilyl[2,2,3,3-$^2H_4$]propionate (TSP) in ^2H_2O in a capillary as an internal standard. The spectral width was 33 898.3 Hz with 32 768 data points, which corresponds to a resolution of 1.03 Hz/point. The determined 10° pulse width was 2.2 μs, the acquisition time was 0.967 s, the pulse delay time was 11.8 μs, and the number of scans was 18 000. The assignments of ^{13}C NMR signals of ^{13}C-labeled vitamin B_{12} were carried out on the basis of reported data (*18*, *19*) and our $^{13}C-^1H$ correlation spectroscopy (COSY) analysis.

RESULTS AND DISCUSSION

Calculation of ^{13}C Incorporation Ratios in ^{13}C-Labeled Vitamin B_{12}. P. shermanii forms DMBI, which is biosynthesized from riboflavin, only under aerobic conditions (*16*, *17*). Namely, oxygen is required for the biosynthesis of DMBI. For this feeding experiment under anaerobic conditions, DMBI was added. Therefore, the origin of the DMBI moiety in ^{13}C-labeled vitamin B_{12} biosynthesized from D-[1-^{13}C]glucose is only the added DMBI. The signals of carbons (B-2−B-11) of the DMBI moiety in ^{13}C-labeled vitamin B_{12} show the natural ratio of ^{13}C, and thus can be used as reference signals. Comparison of the signal intensities in the ^{13}C NMR spectrum (Figure 2) of ^{13}C-labeled vitamin B_{12} with those of vitamin B_{12} (**1**) gave the ^{13}C-enrichment ratio for each carbon in ^{13}C-labeled vitamin B_{12}, as summarized in Table 1.

Biosynthetic Pathways Leading to ALA in P. shermanii. The corrin ring moiety of vitamin B_{12} (**1**) is derived from all the carbons of ALA (**2**), which may be formed via the Shemin pathway and/or the C5 pathway (Figure 1), and the methyl carbon of L-methionine (*20*−*26*). As shown in Table 1, the average ^{13}C-enrichment ratio of 27, 32, 38, 43, 50, 57, and 61 [derived from C-1 of ALA (**2**)] is 3.4-fold, that of 26, 31, 37, 42, 47, 49, 56, and 60 [derived from C-2 of ALA (**2**)] is 7.7-fold, that of 2, 7, 12, 18, 30, 41, 48, and 55 [derived from C-3 of ALA (**2**)] is 7.6-fold, that of 1, 3, 6, 8, 11, 13, 17, and 19 [derived from C-4 of ALA (**2**)] is 6.8-fold, that of 4, 5, 9, 10, 14, 15, and 16 [derived from C-5 of

Table 1: ^{13}C-Enrichment Ratios for Carbon Atoms in ^{13}C-Labeled Vitamin B_{12} Derived from D-[1-^{13}C]Glucose in *P. Shermanii*[a]

5,6-Dimethylbenzimidazole Moiety (Reference Signal)[b]								
B-2	B-4	B-5	B-6	B-7	B-8	B-9	B-10, B-11	
144.55	119.18	137.84	135.76	114.19	132.66	139.42	22.60, 33.09	
1.0	1.1	1.3	0.8	1.1	0.9	0.9	1.3, 0.8	
Corrin Ring Moiety[c]								
C-1[d]	27	32	38	43		50	57	61
	178.50	180.50	177.80	179.75		180.82	177.46	178.38
	3.7	3.7	3.1	3.6		3.1	3.3	3.1
C-2[e]	26	31	37	42	47	49	56	60
	45.49	37.62	45.64	34.18	34.00	37.31	35.26	34.32
	7.4	8.0	8.0	7.9	7.9	7.4	7.7	7.5
C-3[f]	2	7	12	18	30	41	48	55
	49.95	54.19	50.86	41.74	28.72	28.65	30.73	34.95
	7.6	7.2	7.6	7.8	7.6	7.3	7.5	7.9
C-4[g]	1	3	6	8	11	13	17	19
	87.83	59.03	168.01	58.44	179.62	56.40	61.92	77.65
	6.7	6.6	7.0	6.8	7.1	7.0	6.6	6.5
C-5[h]		4	5	9	10	14	15	16
		182.75	110.26	176.27	97.64	168.75	106.82	181.63
		1.9	2.2	2.1	1.8	1.9	1.7	1.8
methyl[i]	20	25	35	36	46	53	54	
	22.00	21.73	18.10	18.57	21.93	17.87	19.51	
	1.2	1.3	1.4	1.3	1.2	1.2	1.1	
D-Ribose Moiety[j]								
R-1		R-2	R-3		R-4		R-5	
89.74		71.52	75.64 (5.2)		84.74 (7.2)		63.07	
40.9		2.0	20.0		2.4		41.2	
Aminopropanol Moiety[j]								
Pr-1		Pr-2			Pr-3			
48.01 (4.7)		75.78 (4.1)			22.07			
4.2		3.6			1.3			

[a] For each group shown in the table, the first line indicates the carbon positions, the second line gives the ^{13}C NMR chemical shift values in parts per million, and the third line shows the ^{13}C-enrichment ratio. [b] For details of calculation of ^{13}C-incorporation ratios in ^{13}C-labeled vitamin B_{12}, see Results and Discussion. The average ^{13}C-enrichment ratio for the DMBI moiety is 1.0. [c] Carbons of the corrin ring moiety are classified into six groups according to their biosynthetic origin: C-1−C-5 are carbons of ALA (**2**), and methyl indicates the methyl carbon of L-methionine. [d] Average ^{13}C-enrichment ratio for C-1 is 3.4. [e] Average ^{13}C-enrichment ratio for C-2 is 7.7. [f] Average ^{13}C-enrichment ratio for C-3 is 7.6. [g] Average ^{13}C-enrichment ratio for C-4 is 6.8. [h] Average ^{13}C-enrichment ratio for C-5 is 1.9. [i] Average ^{13}C-enrichment ratio for the methyl carbon of L-methionine is 1.2. [j] Values in parentheses are coupling constants, in hertz, for doublet signals.

ALA (**2**)] is 1.9-fold, and that of 20, 25, 35, 36, 46, 53, and 54 (derived from the methyl carbon of L-methionine) is 1.2-fold. The C-1−C-5 carbons of ALA (**2**) were labeled, and the methyl carbon of L-methionine was not labeled with ^{13}C from D-[1-^{13}C]glucose.

Figure 3 shows the positions that are predicted to be labeled in ALA (**2ii-4ii**−**2vii-4vii** and **2i-6i**−**2v-6v**) biosynthesized from ^{13}C-labeled succinyl-CoA (**4ii**−**4vii**) and ^{13}C-labeled α-ketoglutaric acid (**6i**−**6v**) via the Shemin pathway and the C5 pathway. ALA (**2**) labeled with ^{13}C on C-1 appears via the Shemin pathway, never via the C5 pathway, and ALA (**2**) labeled with ^{13}C on C-5 appears via the C5 pathway, never via the Shemin pathway. Therefore, the observed ^{13}C-enrichment at carbons of ^{13}C-labeled vitamin B_{12} derived from C-1 and C-5 of ALA (**2**) suggests that both pathways to ALA (**2**) may operate in *P. shermanii*.

As shown in Figure 3, the biosynthesis of ALA molecules (**2iv-4iv** and **2v-6v**) labeled with ^{13}C on C-1 and C-5 can be rationalized as follows. Succinyl-CoA, which is formed in the second cycle of the TCA cycle, is labeled with ^{13}C on C-1 at the first entry of [2-^{13}C]acetyl-CoA (**7i**) into the TCA cycle and transformed to succinic acid. At this time, succinic acid molecules labeled with ^{13}C on C-4 and C-1 appear in equal quantity. Succinic acid labeled with ^{13}C on C-4 and C-1 can revert to succinyl-CoA (**4iv** and **4v**), affording succinyl-CoA (**4iv** and **4v**) labeled with ^{13}C on C-4 and C-1 in equal quantity. Part of succinyl-CoA (**4iv** and **4v**) labeled with ^{13}C on C-4 and C-1 goes into the Shemin pathway and condenses with glycine (**3**). ALA (**2iv-4iv**) labeled with ^{13}C on C-1 is biosynthesized from succinyl-CoA (**4iv**) labeled with ^{13}C on C-4 and affords 3.4-fold ^{13}C-enrichment in ^{13}C-labeled vitamin B_{12}. ALA (**2v-4v**) labeled with ^{13}C on C-4 is concomitantly biosynthesized from succinyl-CoA (**4v**) labeled with ^{13}C on C-1. The rest of succinyl-CoA (**4iv** and **4v**) labeled with ^{13}C on C-4 and C-1 re-enters the TCA cycle and generates ^{13}C-labeled α-ketoglutaric acid (**6iv** and **6v**) via ^{13}C-labeled succinic acid, ^{13}C-labeled oxaloacetic acid, ^{13}C-labeled citric acid, and other ^{13}C-labeled intermediates. ^{13}C-Labeled glutamic acid, which is formed from ^{13}C-labeled α-ketoglutaric acid (**6iv** and **6v**), goes into the C5 pathway and generates ^{13}C-labeled ALA (**2iv-6iv** and **2v-6v**). Namely, succinyl-CoA (**4v**) labeled with ^{13}C on C-1 generates ALA (**2v-6v**) labeled with ^{13}C on C-5 via α-ketoglutaric acid (**6v**) labeled with ^{13}C on C-1, and ^{13}C on C-4 of succinyl-CoA (**4iv**) labeled at the first entry of [2-^{13}C]acetyl-CoA (**7i**) into the TCA cycle disappears from ^{13}C-labeled ALA (**2iv-6iv**). The ^{13}C-enrichment ratio of C-5 of ^{13}C-labeled ALA (**2v-6v**) is decreased in comparison with that of C-1 of ^{13}C-labeled succinyl-CoA (**4v**) re-entered the TCA cycle owing to the many pathways leaving from the pathway between

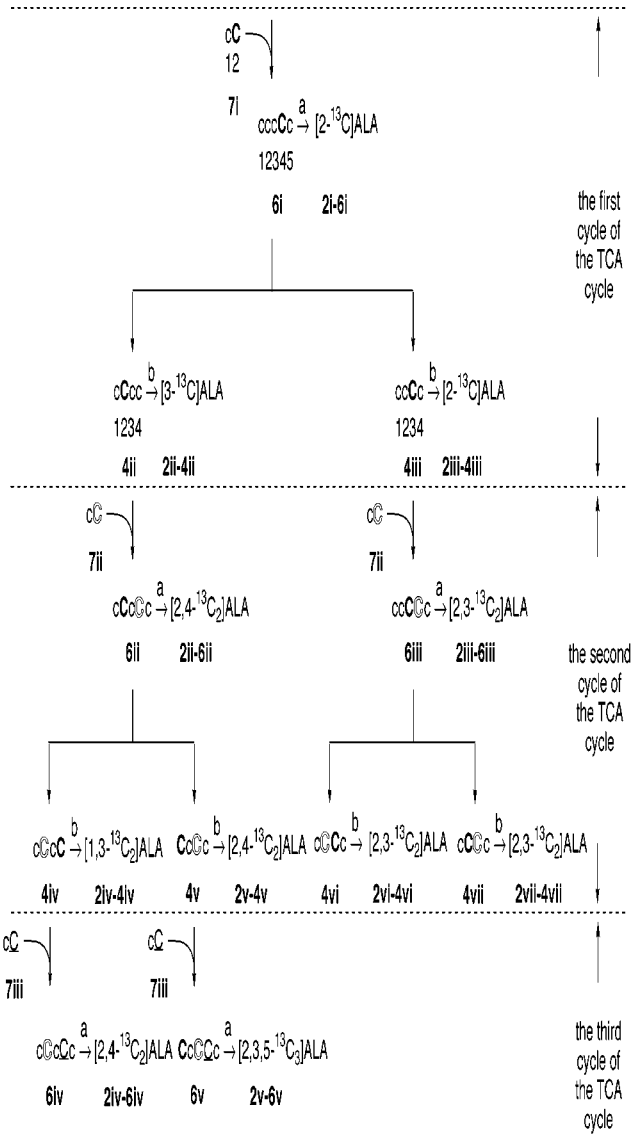

FIGURE 3: Changes of ^{13}C label position during the biosynthesis of ALA (**2ii-4ii**−**2vii-4vii** and **2i-6i**−**2v-6v**), through the Shemin pathway or the C5 pathway via the TCA cycle from [2-^{13}C]acetyl-CoA (**7i**−**7iii**) derived from D-[1-^{13}C]glucose. (ccccc) represents α-ketoglutaric acid (**6i**−**6v**), (cccc) represents succinyl-CoA (**4ii**−**4vii**), and (cc) represents acetyl-CoA (**7i**−**7iii**). (c) is unlabeled carbon, (**C**) is [^{13}C]carbon from the first entry of [2-^{13}C]acetyl-CoA (**7i**) into the TCA cycle, (C) is [^{13}C]carbon from the second entry of [2-^{13}C]acetyl-CoA (**7ii**) into the TCA cycle, and (C) is [^{13}C]carbon from the third entry of [2-^{13}C]acetyl-CoA (**7iii**) into the TCA cycle. ^{13}C-Labeled positions of succinyl-CoA (cccc) (**4ii**−**4vii**) are those of the product formed by reversion from succinic acid. Numbers shown under (ccccc), (cccc), and (cc) are the carbon numbers of the compounds. ^{13}C-Labeled positions of ALA (**2ii-4ii**−**2vii-4vii** and **2i-6i**−**2v-6v**) formed via the C5 pathway from each ^{13}C-labeled (ccccc) and via the Shemin pathway from each ^{13}C-labeled (cccc) are shown at the side. (a) and (b) on arrows (→) show the C5 pathway and the Shemin pathway, respectively.

succinyl-CoA (**4**) and glutamic acid (**5**), and ALA (**2v-6v**) labeled with ^{13}C on C-5 affords at least 1.9-fold ^{13}C-enrichment in ^{13}C-labeled vitamin B_{12}.

On the basis of the putative biosynthetic pathways of ALA (**2iv-4iv** and **2v-6v**) labeled with ^{13}C on C-1 and C-5, the ^{13}C-enrichment ratio of carbons in ^{13}C-labeled vitamin B_{12} derived from C-1 of ALA (**2iv-4iv**) should reflect the ratio of ALA biosynthesis from the Shemin pathway, and the ^{13}C-enrichment ratio of carbons in ^{13}C-labeled vitamin B_{12} derived from C-5 of ALA (**2v-6v**) should reflect the ratio of ALA biosynthesis from the C5 pathway. Thus, on the assumption that substantial scrambling of the label does not occur (see below), the ratio of the ratio of ALA biosynthesis from the Shemin pathway to that from the C5 pathway takes the value of at most 1.8 (= 3.4/1.9). Further, the ratio of ALA biosynthesis from the Shemin pathway is larger than that from the C5 pathway, as similar ^{13}C-enrichment ratios (7.7- and 7.6-fold) of carbons in ^{13}C-labeled vitamin B_{12} derived from C-2 and C-3 of ALA (**2**) are found in Table 1. If the ratio of ALA biosynthesis from the C5 pathway is larger than that from the Shemin pathway, the ^{13}C-enrichment ratio of carbons in ^{13}C-labeled vitamin B_{12} derived from C-2 of ALA (**2**) should be much larger than that from C-3 of ALA (**2**), based on the relation of [2-^{13}C]ALA (**2i-6i**) biosynthesized via the first C5 pathway and [3-^{13}C]- and [2-^{13}C]ALA (**2ii-4ii** and **2iii-4iii**) biosynthesized via the first Shemin pathway with high ^{13}C enrichment (Figure 3). Thus, we can estimate the relative contributions of the Shemin pathway and the C5 pathway to ALA biosynthesis as $1 <$ [(ratio of ALA biosynthesis from the Shemin pathway)/(that from the C5 pathway)] < 1.8.

In a study such as this, it is essential to consider the extent of scrambling of the label due to a range of possible alternative or competing biosynthetic pathways or degradative reactions, particularly since a relatively long culture period (7 days) was employed. Although we cannot assess the importance of all the possible reactions, the contribution of the second passage through the TCA cycle, which is likely to be one of the major contributors to label scrambling, can be evaluated from our observations. That is, there is a contribution to the biosynthesis of ALA, which would be labeled with ^{13}C on C-1 and C-5, from [2-^{13}C]acetyl-CoA (**7ii**) generated in the second cycle of the TCA cycle (shown as cC). Since this results in the synthesis of ALA (**2iii-6iii**, **2vi-4vi**, and **2vii-4vii**) with adjacent labeled carbons at C-2 and C-3 (Figure 3), we can estimate the contribution of [2-^{13}C]acetyl-CoA (**7ii**) from the second turn of the TCA cycle from the ratio of doublet and singlet signals in the ^{13}C NMR spectrum; it was concluded to amount to 7−9%. This suggests that extensive scrambling of the label does not occur and that this approach to evaluate the contributions of the two pathways is reasonable.

Biosynthetic Pathways of D-Ribose Moiety of Vitamin B_{12} from D-[1-^{13}C]Glucose in P. shermanii. Three carbons of the D-ribose moiety in ^{13}C-labeled vitamin B_{12} showed very high ^{13}C enrichment, 40.9-fold at R-1, 41.2-fold at R-5, and 20.0-fold at R-3 (Table 1). D-[1-^{13}C]Fructose 6-phosphate and D-[3-^{13}C]glyceraldehyde 3-phosphate, which would be formed from D-[1-^{13}C]glucose by glycolysis, enter the pentose phosphate pathway under anaerobic conditions, and generate D-[1,5-$^{13}C_2$]xylulose 5-phosphate. Then, the major pathways would afford a D-ribose moiety derived from D-[1,5-$^{13}C_2$]xylulose 5-phosphate highly enriched at R-1 and R-5. The ^{13}C-enrichment at R-3 should arise from D-[1,3-$^{13}C_2$]glyceraldehyde 3-phosphate formed from D-[1,5-$^{13}C_2$]-xylulose 5-phosphate and D-[1,5-$^{13}C_2$]ribose 5-phosphate, which are intermediates on the major pathways, via the minor pathways.

*Biosynthetic Pathways of Aminopropanol Moiety of Vitamin B_{12} (**1**) from D-[1-^{13}C]Glucose in P. shermanii.* As shown in Table 1, similar ^{13}C enrichments of 4.2- and 3.6-

fold were seen at Pr-1 and Pr-2. The carbon of Pr-3 was not labeled with ^{13}C from D-[1-^{13}C]glucose. These results can be rationalized in terms of the biopathway via L-[2- or 3-^{13}C]-aspartic acid, L-[2- or 3-^{13}C]threonine, and [1- or 2-^{13}C]-aminopropanol from [2- or 3-^{13}C]oxaloacetic acid generated in the TCA cycle with theoretically the same ^{13}C-enrichment ratio at C-2 and C-3.

CONCLUSION

Our results suggest that ALA (**2**) may be synthesized via both the Shemin pathway and the C5 pathway from the TCA cycle by *P. shermanii* under anaerobic conditions, with the relative contributions being $1 <$ [(ratio of ALA biosynthesis from the Shemin pathway)/(that from the C5 pathway)] < 1.8. Although the influence of label scrambling could not be quantitatively determined, the effect of second passage through the TCA cycle (likely to be a major contributor) was estimated to amount to only 7−9%. We also found that the D-ribose moiety of vitamin B_{12} (**1**) is synthesized via the pentose phosphate pathway, and the aminopropanol moiety of vitamin B_{12} (**1**) is generated from L-[2- or 3-^{13}C]threonine formed via [2- or 3-^{13}C]oxaloacetic acid. Further refinement of this experimental approach may allow a definitive conclusion.

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