Biosynthesis of Vitamin B_6: Origin of Pyridoxine by the Union of Two Acyclic Precursors, 1-Deoxy-D-xylulose and 4-Hydroxy-L-threonine

Isaac A. Kennedy,[1] Robert E. Hill,[2] Richard M. Pauloski,[1] Brian G. Sayer,[1] and Ian D. Spenser*,[1]

Departments of Chemistry and Pathology McMaster University, Hamilton, Ontario, Canada L8S 4M1

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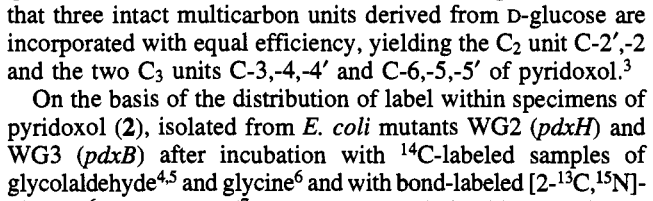
The entire carbon skeleton of vitamin B_6 (pyridoxine, pyridoxol) (**2**) is derived from glucose.[3] Committed intermediates between glucose and the vitamin have not been identified, however. We now present results showing that the C_5 unit C-2′, -2,-3,-4,-4′ and the C_3N unit N-1,C-6,-5,-5′ of pyridoxol are derived from 1-deoxy-D-xylulose (**1**) and 4-hydroxy-L-threonine (**3**), respectively, and that these two compounds serve as intermediates on the route from glucose. The two precursors account for the origin of the entire skeleton of vitamin B_6.



1 **2** **3**

Glucose supplies all of the carbon atoms of pyridoxol, in a characteristic manner.[3] The ^{13}C NMR spectrum of pyridoxol hydrochloride, isolated from *Escherichia coli* B mutant WG2 (*pdxH*) after incubation with D-[1,2,3,4,5,6-$^{13}C_6$]glucose, shows that three intact multicarbon units derived from D-glucose are incorporated with equal efficiency, yielding the C_2 unit C-2′,-2 and the two C_3 units C-3,-4,-4′ and C-6,-5,-5′ of pyridoxol.[3]

On the basis of the distribution of label within specimens of pyridoxol (**2**), isolated from *E. coli* mutants WG2 (*pdxH*) and WG3 (*pdxB*) after incubation with ^{14}C-labeled samples of glycolaldehyde[4,5] and glycine[6] and with bond-labeled [2-^{13}C,^{15}N]-glycine,[6] we suggested[7] that a C_3N unit derived by decarboxylation of 4-hydroxythreonine (**3**) was the progenitor of the C_3N unit N-1,C-6,-5,-5′. This notion was extended by M. E. Winkler on the basis of genetic studies.[8] Further indirect evidence that this amino acid is implicated in the biosynthesis of vitamin B_6 came from nutritional investigations with *E. coli* mutants *pdxB* and *pdxC* (=*SerC*), blocked in the biosynthesis of pyridoxine. These mutants showed a growth response to 4-hydroxy-L-threonine.[9]

The remaining C_5 unit of pyridoxol, C-2′,-2,-3,-4,-4′, is generated by combination of a C_2 unit, C-2′,-2, with a C_3 unit, C-3,-4,-4′, each of which is derived intact from glucose,[3] via the CH_3CO group of pyruvic acid[10] and via a triose,[11] respectively. A likely intermediate on the route from glucose into this C_5 unit is 1-deoxy-D-xylulose (**1**), a compound that has been detected in *E. coli* and other bacteria,[12] where it serves as the precursor of a C_5 unit within thiamin (vitamin B_1).[13] 1-Deoxy-D-xylulose arises by pyruvate dehydrogenase (EC 1.2.4.1) catalyzed acyloin type condensation of D-glyceraldehyde with pyruvic acid, a reaction that is accompanied by decarboxylation.[14] Deuterium-labeled 1-deoxy-D-xylulose entered pyridoxol,[15] affording preliminary evidence that it supplies the C_5 chain C-2′,-2,-3,-4,-4′.

If 1-deoxy-D-xylulose (**1**) were indeed the precursor of the C_5 unit of pyridoxol, C-2′,-2,-3,-4,-4′, then a feeding experiment with D-[$^{13}C_6$]glucose in the presence of unlabeled 1-deoxy-D-xylulose should lead to a reduction of entry of ^{13}C into the C_2 unit C-2′,-2 and the C_3 unit C-3,-4,-4′ compared to the level of ^{13}C labeling within the C_3 unit C-6,-5,-5′, whose derivation from glucose should be unaffected. Conversely, a feeding experiment with D-[$^{13}C_6$]glucose in the presence of unlabeled 4-hydroxy-L-threonine (**3**) would be expected to lead to a lowering of the level of ^{13}C labeling within the C_3 unit C-6,-5,-5′ compared to that within the C_2 unit C-2′,-2 and the C_3 unit C-3,-4,-4′. Importantly, the lowering of the level of labeling within the affected carbon atoms of the sample from each of the two experiments can be determined only by comparison with an internal standard within the same sample. The level of labeling within the carbon atoms of the unaffected unit serves as such an internal standard. Comparison of incorporation levels within different samples, without such an internal standard, is entirely unreliable as a measure of relative incorporation efficiency.

In each of two tracer experiments, five 1-L cultures of *E. coli* B mutant WG2 were each incubated[16] in the presence of D-[1,2,3,4,5,6-$^{13}C_6$]glucose (CIL, 99% ^{13}C, 200 mg) together with nonenriched D-glucose (800 mg) (i.e., 5.5 mM D-glucose/L) and either 1-deoxy-D-xylulose[17] (**1**) (750 mg, 5.5 mM) (experiment 1) or 4-hydroxy-L-threonine[19] (**3**) (750 mg, 5.5 mM) plus L-threonine (20 mg)[21] (experiment 2). Pyridoxol hydrochloride was isolated[16] from each 1-L culture after addition of natural abundance pyridoxol hydrochloride (2.5 mg) as carrier and was purified by column and thin layer chromatography. The products obtained from the five 1-L cultures of experiment 1 were combined, as were those from the five 1-L cultures from experiment 2. The two samples of pyridoxol hydrochloride were then subjected to high-vacuum sublimation as the final step of purification.[16]

The ^{13}C NMR spectrum of the sample of pyridoxol hydrochloride from the experiment with D-[$^{13}C_6$]glucose in the presence of unlabeled 1-deoxy-D-xylulose (experiment 1) (Figure 1A) shows the same pattern of satellites that is present in the spectrum of the sample derived from D-[$^{13}C_6$]glucose alone.

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(17) 1-Deoxy-D-xylulose was synthesized in six steps,[18] starting from (−)-2,3-*O*-isopropylidene-D-threitol ((4*R*,5*R*)-4,5-bis(hydroxymethyl)-2,2-dimethyl-1,3-dioxolane, Aldrich).
(18) To be submitted for publication.
(19) 4-Hydroxy-L-threonine was synthesized in four steps, starting from D-erythrono-1,4-lactone (D-erythronic γ-lactone, Aldrich) by a sequence analogous to that employed for the synthesis of its enantiomer.[20]
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(21) 4-Hydroxy-L-threonine shows antimetabolite properties in *E. coli* and inhibits the growth of *E. coli* B WG2 on a pyridoxal supplemented growth medium.[22] Addition of L-threonine (20 mg/L) permits the mutant to grow in the presence of the 4-hydroxy-L-threonine (750 mg/L) present in the medium.

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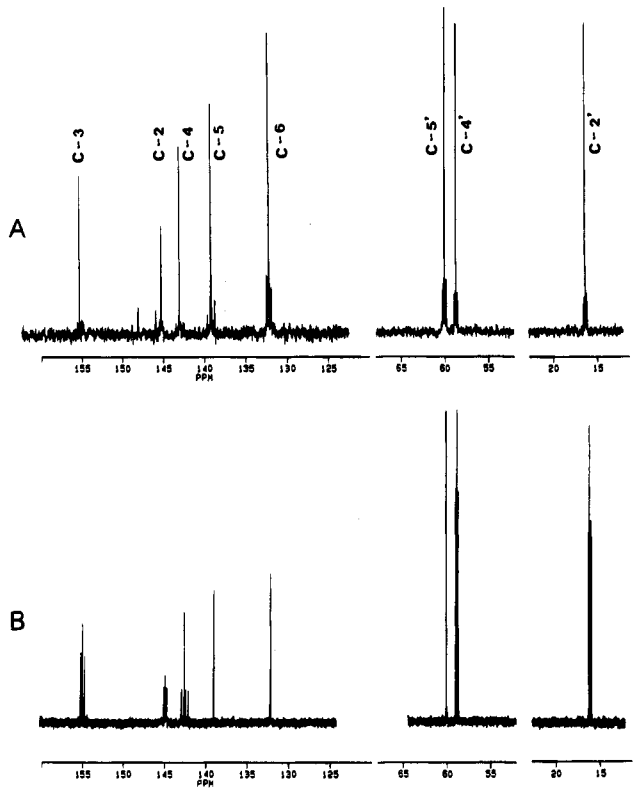


Figure 1. Proton-decoupled ^{13}C NMR spectra of pyridoxol hydrochloride (in 50 μL of D_2O, saturated solution) isolated from *E. coli* B WG2 after incubation with D-[1,2,3,4,5,6-$^{13}C_6$]glucose in the presence of (A) unlabeled 1-deoxy-D-xylulose and (B) unlabeled 4-hydroxy-L-threonine. The spectra were acquired on a Bruker AM 500 spectrometer, operating at 125.776 MHz at 11.74 T. A 90° pulse width (6.4 μs) was used with a spectral width of 29 411 Hz and a recycle time of 7.0 s. The initial memory size was 32 K, which was zero filled to 64 K before Fourier transformation to give a final digital resolution of 0.9 Hz/data point.

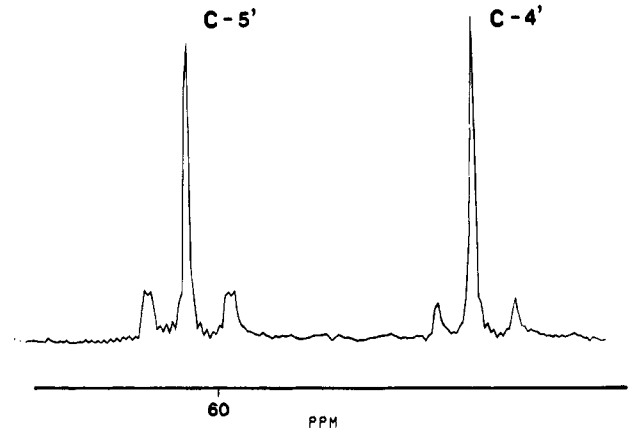
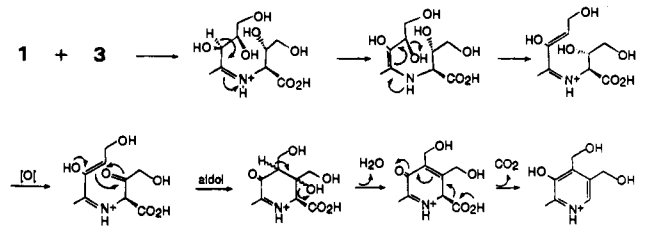


Figure 2. Expanded 58–61 ppm spectral region of the spectrum in Figure 1A.

However, expansion of the spectrum (Figure 2) shows that the area of the satellites of the signal due to C-5′ is approximately 2.5 times greater than that of the C-4′ satellites. Similarly, the area of the satellites of the signal due to C-5 was approximately 3 times greater than that of the C-4 satellites. Thus, the presence of 1-deoxy-D-xylulose has led to a significant reduction of the efficiency of incorporation of label into the C_5 unit C-2′,-2,-3,-4,-4′ relative to the entry of label into the C_3 unit C-6,-5,-5′. Evidently, 1-deoxy-D-xylulose lies on the route from glucose into the C_5 unit C-2′,-2,-3,-4,-4′.

The ^{13}C NMR spectrum of the sample of pyridoxol hydrochloride from the experiment with D-[$^{13}C_6$]glucose in the presence of unlabeled 4-hydroxy-L-threonine (experiment 2)

Scheme 1



(Figures 1B) shows a satellite pattern that is entirely different from that in the spectrum of the sample from D-[$^{13}C_6$]glucose alone. Whereas satellites are observable in the signals due to C-2′, C-2, C-3, C-4, and C-4′, only natural abundance singlets are observable in the signals due to C-6, C-5, and C-5′. Addition of unlabeled 4-hydroxy-L-threonine had inhibited the incorporation of a glucose-derived C_3 unit into the C-6,-5,-5′ segment of pyridoxol, while leaving the entry of glucose-derived fragments into the rest of the molecule unimpaired. Thus, 4-hydroxy-L-threonine lies on the route from D-glucose into the C_3 unit C-6, -5,-5′ of pyridoxol.

The two substrates, 1-deoxy-D-xylulose (**1**) and 4-hydroxy-L-threonine (**3**), account for the origin of the entire skeleton of vitamin B_6. A plausible reaction sequence leading from the two substrates[23] to pyridoxol is shown in Scheme 1.

To attain the ring structure by union of the two substrates, an oxidative step is formally required, and it is therefore of great interest that reducing agents inhibit the biosynthesis of pyridoxol in spinach chloroplasts.[24] The oxidation is here postulated to take place at the carbon atom destined to become C-5 of pyridoxol. An important mechanistic function in the cyclization process is thereby assigned to the hydroxy group that is destined to become the phenolic group at C-3 of pyridoxol.

Only two genes, *pdxA* and *pdxJ*, and therefore only two enzymes, are implicated in the process of ring closure.[25] While it is possible that a single enzyme might catalyze more than one step of the reaction sequence, it is nevertheless reassuring that of the seven steps in Scheme 1 only two, the dehydrogenation and the decarboxylation,[26] are likely to require enzyme catalysis. The remaining steps, Schiff base formation, iminium–enamine equilibrium, elimination of water from a vinylogous carbinolamine and from a site adjacent to a ketone, may well occur spontaneously. Finally, there is precedent for the spontaneous occurrence of an aldol condensation following an enzyme-catalyzed redox reaction, in the formation of dehydroquinic acid.[27]

The results here presented establish that 1-deoxy-D-xylulose and 4-hydroxy-L-threonine represent what are most probably the ultimate acyclic precursors on the route to pyridoxine.[23] The hypothetical reaction sequence (Scheme 1) remains to be substantiated experimentally.

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(23) It has not yet been established whether it is pyridoxol itself or one of its phosphate esters that is the first vitamin B_6 compound to be biosynthesized. Thus, the progenitors of the two "halves" of the molecule could be either the two substrates **1** and **3** or the phosphate ester of one or the other or both. For simplicity, the nonphosphorylated species are shown in Scheme 1.

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(26) The decarboxylation step is arbitrarily shown to occur as the final step in the sequence, as a decarboxylation of a vinylogous β-keto acid. A modified sequence in which decarboxylation of a β-keto acid occurs immediately following the dehydrogenation step is equally plausible.

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