4-Hydroxy-L-threonine, a Committed Precursor of Pyridoxol (Vitamin B_6)

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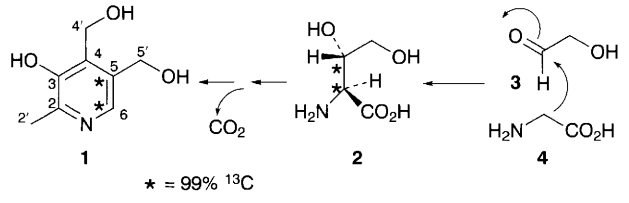
Having established in an earlier experiment that, in *Escherichia coli*, 4-hydroxy-L-threonine [(2*S*,3*S*)-2-amino-3,4-dihydroxybutanoic acid] lies on the route from glucose to vitamin B_6, it is now demonstrated that the amino acid serves as a precursor of pyridoxol, since ^{13}C NMR spectroscopy shows that the intact C-2,3 bond of [2,3-$^{13}C_2$]-4-hydroxy-L-threonine **2** becomes the C-5,6 bond of pyridoxol **1**.

It has been shown by tracer experiments with [1,2-^{14}C]- and with [2-^{14}C]-glycolaldehyde,[1,2] and with [2-^{14}C]- and with [2-^{13}C,^{15}N]-glycine,[3] that, in *Escherichia coli*, C-5 and C-5′ of pyridoxol **1** (unstarred) can be derived from the aldehyde and the carbinol carbon atom, respectively, of glycolaldehyde **3**, and that the N-1,C-6 fragment of the vitamin can be derived from the intact N,C-2 unit of glycine **4**. Based on these findings, we postulated[4] that 4-hydroxy-L-threonine **2** (unstarred), originating from an aldol type condensation of glycine **4** with glycolaldehyde **3**, analogous to the formation of serine from glycine and formaldehyde,[5] catalysed by glycine hydroxymethyltransferase (serine hydroxymethylase, EC 2.1.2.1), might be an intermediate in pyridoxol biosynthesis, to yield the C_3N unit, N-1,C-6,5,5′ of the vitamin. Indirect evidence that 4-hydroxy-L-threonine is implicated in pyridoxine biosynthesis came from genetic[6] and nutritional studies.[7]



The C_3 fragment in question, C-6,5,5′ of pyridoxol, is also generated from an intact C_3 unit that is derived from glucose.[8] We have recently demonstrated[9] that the presence of 4-hydroxy-L-threonine [(2*S*,3*S*)-2-amino-3,4-dihydroxybutanoic acid] in the incubation medium totally inhibits incorporation of the label from [1,2,3,4,5,6-$^{13}C_6$]glucose into C-6,5,5′ of pyridoxol, whereas at the same time incorporation of the label into the other five carbon atoms of pyridoxol remains unimpaired. Thus, 4-hydroxy-L-threonine lies on the pathway from glucose into the C_3 fragment C-6,5,5′ of pyridoxol. Moreover, since 4-hydroxy-L-threonine totally inhibits incorporation of a glucose-derived C_3 fragment into C-6,5,5′ of pyridoxol,[9] it would appear that the amino acid serves as a feedback inhibitor of its own biosynthesis from D-glucose‡ and consequently of this segment of the biosynthetic pathway.

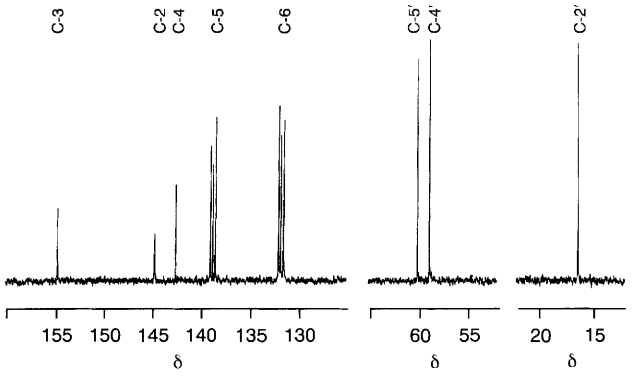


Fig. 1 125.776 MHz proton-decoupled ^{13}C NMR spectrum of pyridoxol hydrochloride (in 50 μl D_2O, saturated solution) isolated from *E. coli* B WG2 after incubation with [2,3-$^{13}C_2$]-4-hydroxy-L-threonine **2**. The spectrum was acquired on a Bruker AM 500 spectrometer. A 90° pulse width (6.4 μs) was used with a spectral width of 29411 Hz and a recycle time of 10.5 s. 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 per data point.

To provide final substantiation of the role of 4-hydroxy-L-threonine in the biosynthesis of vitamin B_6, that is, to show that the compound serves as a direct precursor of the C_3N unit N-1,C-6,5,5′ of the vitamin, it must be demonstrated that the C_3N-unit, N,C-2,3,4 of the amino acid is incorporated, with its C-2–C-3 bond intact. We now present evidence, from an experiment with [2,3-$^{13}C_2$]-4-hydroxy-L-threonine **2**, that this is indeed so.

Five 1 l cultures of *E. coli* B mutant WG2 were each incubated, with D-glucose (0.5 g) as the general carbon source, in the presence of [2,3-$^{13}C_2$]-4-hydroxy-L-threonine **2**§ (160 mg) and L-threonine (20 mg l^{-1}). 4-Hydroxy-L-threonine partially inhibits the growth of *E. coli* B on a pyridoxal supplemented growth medium[10] but addition of L-threonine permits the mutant to grow. Pyridoxol hydrochloride was isolated[11] from each 1 l culture after addition of pyridoxol hydrochloride (2.5 mg) as carrier, and was purified by column and thin layer chromatography. The samples were combined and the product purified by sublimation in a high vacuum.

The proton-decoupled 125.7 MHz ^{13}C NMR spectrum of the sample of pyridoxol **1** hydrochloride so obtained is shown in Fig. 1. Each of the two signals, due to C-5 (δ 138.8) ($^1J_{5,6}$ 64.6 ± 0.2 Hz) and C-6 (δ 131.8) ($^1J_{5,6}$ 64.6 ± 0.2 Hz), appears as a doublet, straddling the natural abundance singlet originating from the carrier pyridoxol that had been added to facilitate isolation. Thus, the labelled bond, C-2–C-3 of the precursor **2** had been incorporated intact into the C-6–C-5 bond of pyridoxol **1**. The signals due to the other six carbon atoms appear as natural abundance singlets. Incorporation had been site specific. This finding supplies the missing link in the evidence that 4-hydroxy-L-threonine, originating either from glucose[6,8,9] or from glycine[3] plus glycolaldehyde,[1,2] serves as direct precursor of the C_3N unit N-1,C-6,5,5′ of vitamin B_6.

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Footnotes

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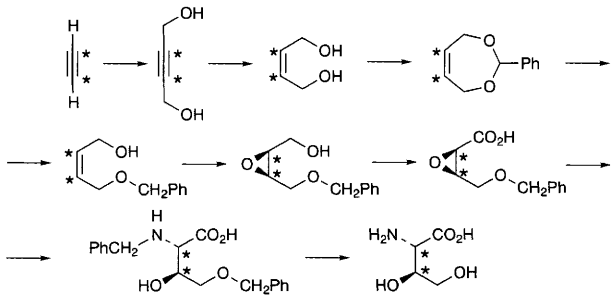
‡ Evidence from genetic studies[6] with pyridoxine-requiring mutants of *Escherichia coli* suggests that the route from glucose into 4-hydroxy-L-threonine proceeds by way of the pentose phosphate pathway and that erythrose 4-phosphate, erythronic acid 4-phosphate and the corresponding α-keto acid are intermediates between glucose and the amino acid.

§ [2,3-$^{13}C_2$]-4-Hydroxy-L-threonine **2** was synthesised from [1,2-$^{13}C_2$]acetylene in 8 steps (Scheme 1), in an overall yield of 13%. A full account of the synthesis has been submitted for publication.



Scheme 1 * = 99% ^{13}C

References

1 R. E. Hill, P. Horsewood, I. D. Spenser and Y. Tani, *J. Chem. Soc., Perkin Trans. 1*, 1975, 1622.
2 G. J. Vella, R. E. Hill, B. S. Mootoo and I. D. Spenser, *J. Biol. Chem.*, 1980, **255**, 3042.
3 A. Iwanow, R. E. Hill, B. G. Sayer and I. D. Spenser, *J. Am. Chem. Soc.*, 1984, **106**, 1840.
4 R. E. Hill and I. D. Spenser, in *Vitamin B_6, Pyridoxal Phosphate; Chemical, Biochemical and Medical Aspects, Part A*, ed. D. Dolphin, R. Poulson and O. Avramovic, Wiley, New York, 1986, pp. 417–476.
5 P. M. Jordan and M. Akhtar, *Biochem. J.*, 1970, **116**, 277.
6 H.-M. Lam and M. E. Winkler, *J. Bacteriol.*, 1990, **172**, 6518.
7 C. D. Drewke, C. Notheis, U. Hansen, E. Leistner, T. Hemscheidt, R. E. Hill and I. D. Spenser, *FEBS Lett.*, 1993, **318**, 125.
8 R. E. Hill, B. G. Sayer and I. D. Spenser, *J. Chem. Soc., Chem. Commun.*, 1986, 612.
9 I. A. Kennedy, R. E. Hill, R. M. Pauloski, B. G. Sayer and I. D. Spenser, *J. Am. Chem. Soc.*, 1995, **117**, 1661.
10 J. W. Westley, D. L. Pruess, L. A. Volpe, T. C. Demny and A. Stempel, *J. Antibiot.*, 1971, **24**, 330.
11 R. E. Hill, F. J. Rowell, R. N. Gupta and I. D. Spenser, *J. Biol. Chem.*, 1972, **247**, 1869.