# Direct Observation of Porphyrinogen Biosynthesis in Living Cells by $^{13}C$ N.M.R. Spectroscopy

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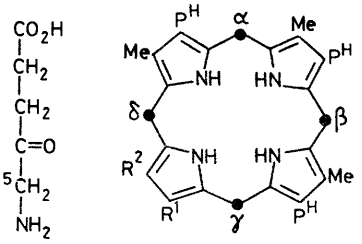
*Summary* The metabolism of $^{13}C$-labelled substrates and of glucose has been observed directly in live cells by proton-decoupled $^{13}C$-Fourier transform n.m.r. spectroscopy.

THE feasibility of using an enriched carbon-13 substrate to study metabolism in the intact cell has been demonstrated in the case of a primary pathway[1] (glycolysis), but to date the method has not been applied to specific solutions to problems in the area of secondary metabolism. The observation of intracellular and/or exogenous intermediates could provide crucial information regarding the dynamics of biosynthesis during the transfer of enzyme-free species in the metabolic machinery. We have studied several pathways in bacterial biosynthesis and now report on the application of this technique in the biosynthesis of the type III-porphyrinogens[2] in *Rhodopseudomonas spheroides* and *Propionibacterium shermanii*.

Packed wet cells of *R. spheroides* (1 g) were incubated in a standard 10 mm n.m.r. tube with phosphate buffer (1·0 ml) in the presence of 5-[$^{13}C$]aminolevulinic acid[3] (ALA) (**1**).

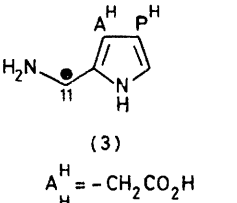



(1)




(2)

a, $R^1 = P^H$, $R^2$ = Me
b, $R^1$ = Me, $R^2 = P^H$




(3)

$A^H = -CH_2CO_2H$
$P^H = -CH_2CH_2CO_2H$

The $^{13}C$ n.m.r. spectra, shown in Figures 1(A) and 1(B), were then recorded over 16 h and clearly reveal the formation of copro'gen III (**2a**) and I (**2b**) (75%/25%)† [Figure 1(B)]. Owing to their sensitivity to light and air, porphyrinogens are not normally isolated but are routinely analysed by oxidation to the corresponding porphyrin.[4] The multiplets at 21·9 and 125·0 p.p.m. ($J$ 51·5 Hz) are, in fact, due to both

† Determined by h.p.l.c. analysis of the copro-methyl esters.

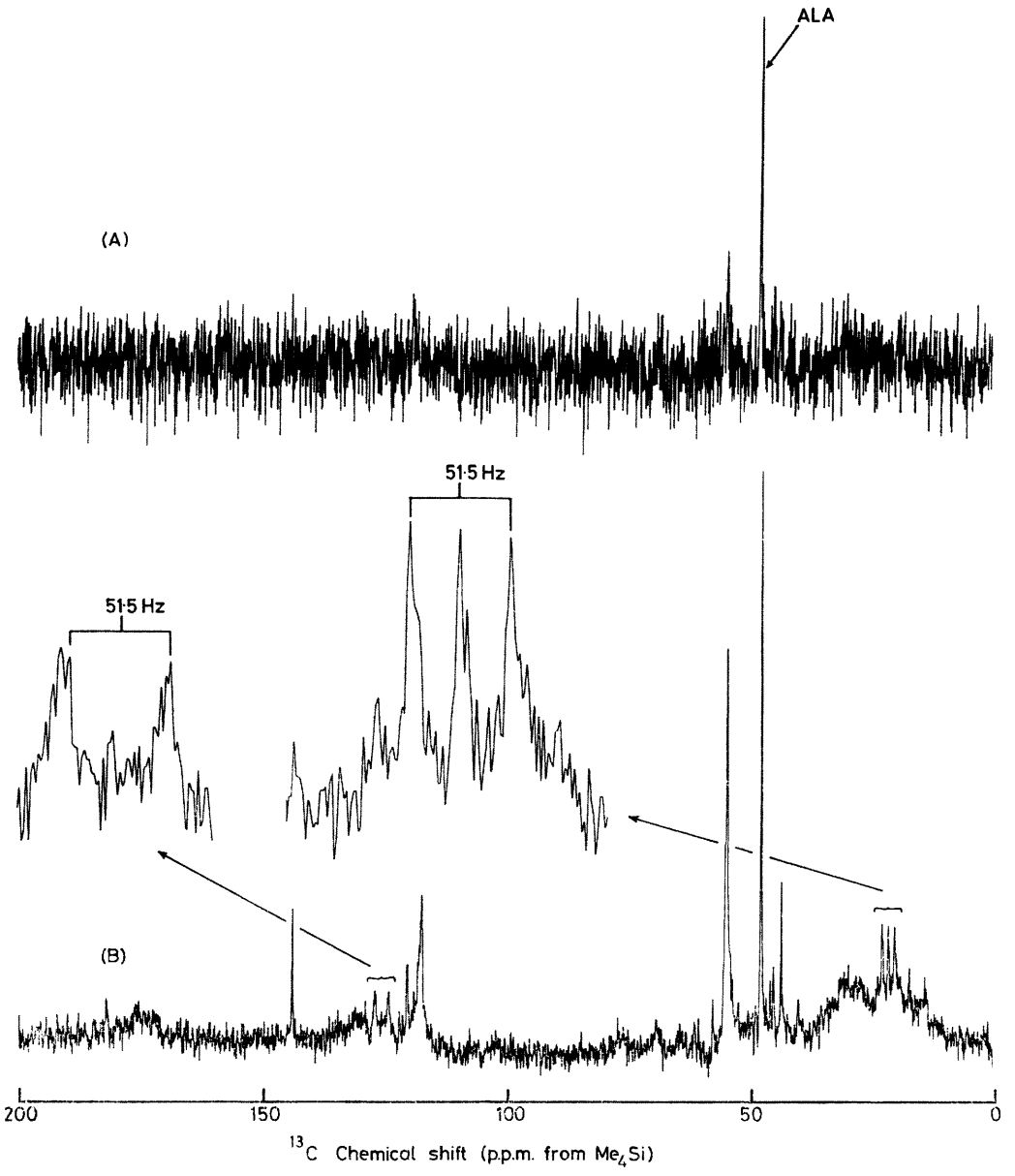



FIGURE 1. 20 MHz $^{13}$C N.m.r. spectrum of *R. spheroides* whole cells incubated with 5-[$^{13}$C]-ALA (**1**) under complete proton noise decoupling conditions. Both spectra were run at 30 °C using 90° pulses, a spectral width of 5,000 Hz, an 8 K data table, a 0·8 pulse repetition rate, and *ca.* 0·7 Hz of line broadening due to exponential weighting of the free induction decay (F.I.D.). Internal lock on 10% $D_2O$ was used. (A) 900 Pulses taken at initial mixing time. (B) 70,713 Pulses beginning 2·8 h after mixing and lasting 16·1 h.

intracellular and extracellular copro'gen, as shown by the appearance of these signals in the spectra of both the supernatant and the washed, resuspended cells run under identical conditions after centrifugation. The use of 11-[$^{13}$C]-PBG[5] (**3**) in the same metabolic pathway, giving rise to a differently labelled species of copro'gen III (**2a**), was next applied to resting cells (1·0 ml) of *P. shermanii* in a glucose supplemented phosphate buffer. The decrease in PBG concentration was followed over an 80 h incubatino in the n.m.r. tube, and the spectra shown in Figures 2(A)—(C) were obtained. In this experiment two metabolic pathways were viewed simultaneously, since glucose (non-enriched) was added to the medium. The formation of propionic acid (Me, 10·9; $CH_2$, 31·0 p.p.m.; both pH dependent) and acetic acid (Me, 23·9 p.p.m.; pH dependent) can easily be discerned even at natural abundance as the glucose concentration falls, whilst porphyrinogen biosynthesis from PBG was monitored by following the growth of intensity of

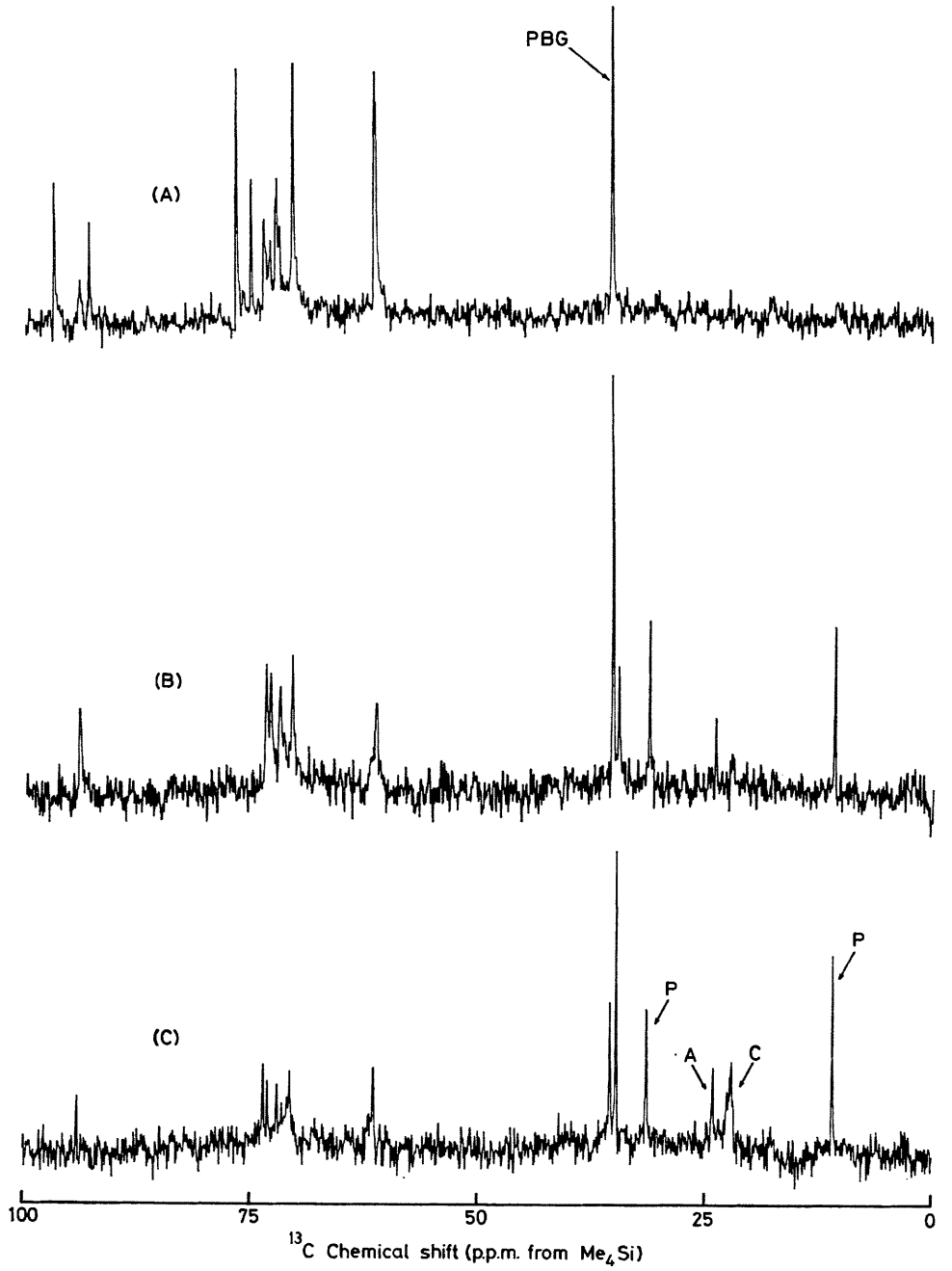



FIGURE 2. 20 MHz $^{13}$C N.m.r. spectrum of *P. shermanii* cells incubated with 11-[$^{13}$C]-PBG (**3**). Instrumental conditions are as noted in Figure 1, except that each spectrum is the result of 15,000 pulses. (A) Initial incubation mixture. (B) 30·7 h Incubation period. (C) 81·8 h Incubation (P: propionic acid; A: acetic acid; C: copro'gen). The pH was periodically adjusted with 10% sodium carbonate.

the broad signal at 22·0 p.p.m.‡ and the complementary intensity decrease of the substrate (δ 34·9 p.p.m.). This direct $^{13}$C n.m.r. technique offers an attractive alternative to previous analyses (u.v., n.m.r., i.r., and chromatography) performed at the porphyrin oxidation level.[3]‡

Obvious and diverse applications of this method to problems in primary and secondary metabolism in bacteria, fungi, plant, and mammalian cells are under investigation. Of special interest are those pathways where only small 'building blocks,' *e.g.* amino acids, but not oligo-peptides, are capable of penetrating the cell wall since their dynamic relationships can now be followed inside and outside the cell.

‡ As far as we are aware, this is the first recorded $^{13}$C n.m.r. spectrum of a natural porphyrinogen. The signal at 22 p.p.m. is assigned to the α–δ *meso* methylene groups of (**2a**). Note that the multiplicity of this signal in Figure 1(B) is due to coupling with the adjacent $sp^2$ carbons (125 p.p.m.) which are also enriched from 5-[$^{13}$C]-ALA.

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[1] R. T. Eakin, L. O. Morgan, E. T. Gregg, and N. Matwiyoff, *F.E.B.S. Letters*, 1972, **28**, 259; K. Ugurbil, T. R. Brown, J. A. den Hollander, P. Glynn, and R. G. Schulman, *Proc. Nat. Acad. Sci. U.S.A.*, 1978, **75**, 3742.
[2] A. R. Battersby and E. McDonald, *Phil. Trans.*, 1976, **273**, 161.
[3] A. R. Battersby, E. Hunt, E. McDonald, and J. Moron, *J.C.S. Perkin I*, 1973, 2917.
[4] J. H. Furhop and K. Smith, in 'Porphyrins and Metalloporphyrins,' ed. K. Smith, Elsevier, Amsterdam 1975, p. 793.
[5] Obtained by a modification of the method in ref. 4, p. 758.