# In vivo proton NMR spectroscopy

## Use of a reverse-POMMIE sequence to monitor formaldehyde detoxification in *Escherichia coli*

David M. Doddrell, Kathryn M. Nicholls[+] and Jeremy K.M. Sanders[+]*

*School of Science, Griffith University, Nathan 4111, Australia,* and
[+]*University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW, England*

Received 27 February 1984; revised version received 14 March 1984

The metabolism of [$^{13}$C]formaldehyde in *E.coli* has been observed by proton NMR; the reverse-POMMIE pulse sequence, which saturates all $^{1}$H signals and then transfers magnetisation from $^{13}$C to $^{1}$H, suppresses water by $10^5$-fold, allowing observation of metabolism at the millimolar level

*$^{1}$H NMR* *In vivo NMR* *Formaldehyde* *Metabolism* *Detoxification* E.coli

## 1. INTRODUCTION

The use of in vivo $^{13}$C NMR spectroscopy to follow the metabolism of $^{13}$C-enriched substrates is well established. The corresponding observation of proton metabolism is more difficult. Macromolecular and water resonances can be suppressed in various ways [1,2] but the substrate cannot be enriched with protons and so neither it nor its metabolites can be easily distinguished from the numerous natural small molecule cellular components. In favourable cases, $^{2}$H NMR can be used to follow metabolism of deuterated substrates [3] but the method has severe limitations.

Several methods for observing protons coupled to $^{13}$C have been reported [4–8]. These are based on spin-decoupling [4], spin-echoes [5,6] or reverse polarisation transfer [7,8]; however, none has achieved sufficient water suppression to allow observation of millimolar biochemistry. We show here that the reverse-POMMIE [9] sequence readily gives $10^5$-fold water suppression, and we illustrate the technique using the detoxifying metabolism of formaldehyde by *Escherichia coli* [3]. This is a particularly demanding application as the formaldehyde hydrate resonance occurs at 4.8$\delta$, essentially coincident with water.

POMMIE (Phase Oscillation to MaxiMIse Editing) is closely related to other polarisation transfer sequences such as INEPT [7] and DEPT [8]. It involves polarisation transfer from $^{1}$H to $^{13}$C and exploits the particular phase properties of the multiple quantum state of a coupled $^{13}$C-$^{1}$H pair; by means of suitable pulse and phase cycling it detects only those $^{13}$C nuclei which are coupled to $^{1}$H. The corresponding reverse sequences for INEPT, DEPT and POMMIE transfer polarisation from $^{13}$C to $^{1}$H and so ideally they detect only those protons which are coupled to $^{13}$C. In practice, POMMIE appears to be the most efficient sequence to date.

## 2. MATERIALS AND METHODS

*E.Coli* was grown, prepared for spectroscopy and challenged with $^{13}$C-labelled formaldehyde as in [3]. The solvent used for NMR spectroscopy was 4:1 $H_2O$:$D_2O$. Spectra of whole cell suspensions were obtained without sample spinning. Supernatant solutions containing metabolites were obtained

* To whom correspondence should be addressed

*Published by Elsevier Science Publishers B.V.*
00145793/84/$3.00 

by centrifugation of bacterial suspensions which had been previously challenged with [$^{13}$C]formaldehyde; spectra of supernatants were acquired with sample spinning. $^1$H spectra were obtained at 200 MHz on an extensively modified Bruker spectrometer using the reverse-POMMIE [9] sequence:

$$\text{spherical decoupling} - \underset{P_1}{\frac{\pi}{2}}[C,\pm x] - \frac{1}{2J} - \underset{P_2}{\frac{\pi}{2}}[H,i]\underset{P_3}{\frac{\pi}{2}}[H,\psi]\underset{P_4}{\pi}[C] - \frac{1}{2J} - \underset{P_5}{\frac{\pi}{2}}[C,y]\underset{P_6}{\pi}[H] - \frac{1}{2J} - \text{acquire } {}^1\text{H} \pm \text{receiver}$$

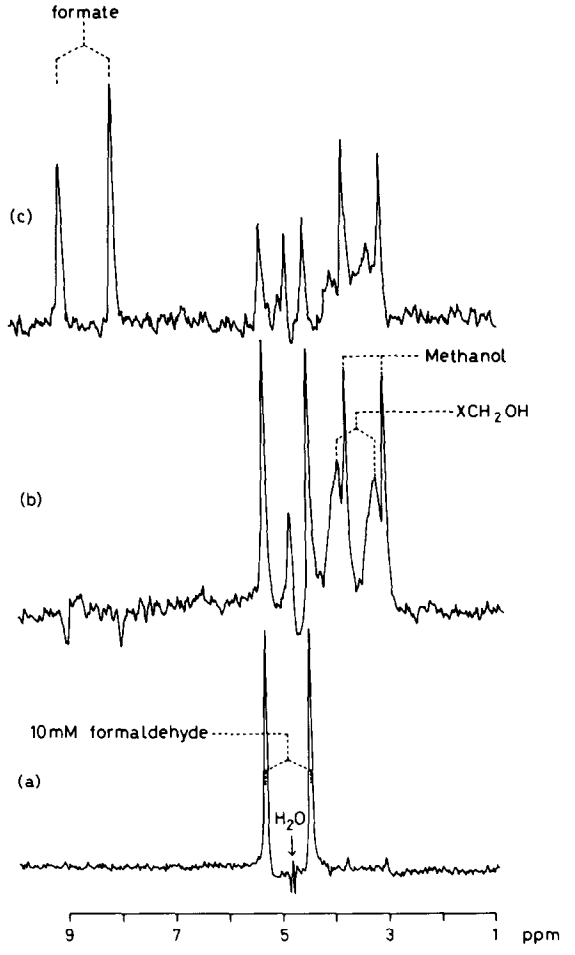


Fig.1. 200 MHz $^1$H reverse-POMMIE spectra of an *E.coli* suspension in a 4:1 $H_2O$:$D_2O$ mixture (a) acquired immediately after addition of [$^{13}$C]formaldehyde to give a concentration of 10 mM; (b) and (c) after some hours of metabolism, with $(2J)^{-1}$ set to 3.4 and 2.5 ms, respectively.

A 3 s spherical decoupling period was used to presaturate all $^1$H signals and to build up nOe at $^{13}$C. Further suppression of non-$^{13}$C-coupled protons was achieved by alternation of both the $P_1$ pulse and receiver phase. The multiple quantum read pulse $P_3$ was fixed with a 45° phase shift. The multiple quantum formation pulse $P_2$ was set at $+x$ or $-x$ depending on the relative phases desired for the CH, $CH_2$ and $CH_3$ signals [9]. $(2J)^{-1}$ was set to 3.4 or 140 ms to optimise observation of multiplets with $J_{CH}$ of 140 and 200 Hz, respectively. In metabolic experiments, 256 transients requiring a total acquisition time of 30 min were acquired. Spectra were generally processed with 2 Hz line broadening.

## 3. RESULTS

Fig.1(a) shows the 200 MHz reverse-POMMIE $^1$H spectrum of 10 mM formaldehyde hydrate, 90% $^{13}$C-labelled, in an aqueous suspension of *E.coli*. This spectrum was acquired immediately after formaldehyde addition and shows essentially only the formaldehyde doublet ($J_{CH}$ = 162 Hz) plus some methanol which is the metabolic reduction product; the residual water signal at 4.8$\delta$, corresponding to 80 M protons, is suppressed approx. $10^5$-fold. After some metabolism had occurred it was straightforward, using $(2J)^{-1}$ = 3.4 ms (fig.1b, $J \sim 140$ Hz),

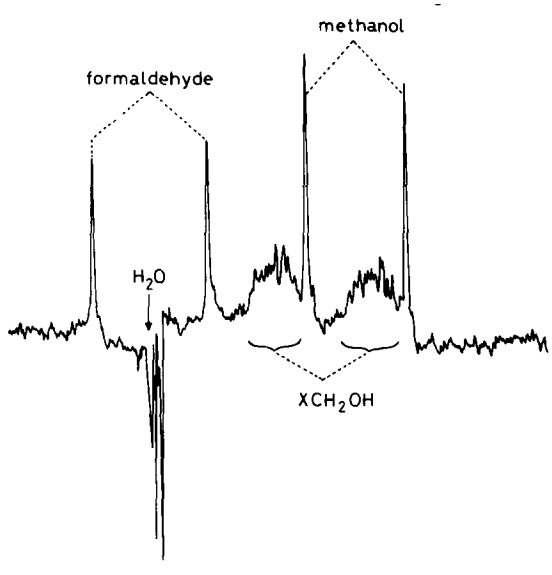


Fig.2. Higher resolution reverse-POMMIE spectrum [$(2J)^{-1}$ = 3.4 ms] of a supernatant after partial formaldehyde metabolism.

to detect methanol and unidentified metabolites $XCH_2OH$ and, by changing $(2J)^{-1}$ to 2.5 ms, to optimise the formate signal (fig.1c, $J = 197$ Hz). Extended data acquisition [$(2J)^{-1} = 3.4$ ms] on a supernatant sample gave a higher resolution spectrum (fig.2). The complex signals at 3.3–3.9 ppm arise from overlapping and tightly coupled protons of 3 separate metabolites, all $XCH_2OH$ [3]. Reversal of the phase of the $P_2$ pulse also reversed the phase of the methanol signal, confirming it as belonging to a $CH_3$ group [9].

Repeated additions of formaldehyde led to further detoxification of the drug as reported in [3].

## 4. DISCUSSION

A $10^5$-fold suppression of water is achieved in the reverse-POMMIE sequence in two stages. The first consists of presaturation using a spherical randomisation field. The second relies on a phase alternation of the signals from protons attached to $^{13}C$ as a result of phase alternating $P_1$; alternating the receiver phase then gives coherent addition of $^1H$-$^{13}C$ signals but subtraction of alternate water signals. In this way it is possible to observe millimolar biochemistry even with signals which are extremely close to the water resonance.

In metabolic experiments these 200 MHz $^1H$ spectra have better sensitivity than our earlier $^{13}C$ observations at much higher fields [3]. Given a suitable probe, the intrinsic sensitivity should be 75% that of normal $^1H$ NMR. These spectra are more informative than conventional $^{13}C$ spectra as they contain both H-H and H-C couplings. The extremely complex overlapping signals of the $XCH_2OH$ metabolites can be separated if necessary by suitable two-dimensional experiments or by a carbon-selective version of the present technique. It is clear that, even allowing for the need for $^{13}C$-enrichment and the dependence on $J$(C-H), this is a powerful tool for in vivo NMR spectroscopy.

Our results confirm the presence of the previously described formaldehyde detoxification in *E.coli* and confirm the metabolite proton chemical shifts which were previously inferred indirectly [3].

## ACKNOWLEDGEMENTS

We are grateful to SERC (K.M.N.) and the Royal Society of Chemistry (J.K.M.S.) for financial support.

## REFERENCES

[1] Nicholson, J.K., O'Flynn, M.P., Sadler, P.J., Macleod, A.F., Juul, S.M. and Sönksen, P.H. (1984) Biochem. J. 217, 365–375.
[2] Arus, C., Barany, M., Westler, W.M. and Markley, J.L. (1984) FEBS Lett. 165, 231–237.
[3] Hunter, B.K., Nicholls, K.M. and Sanders, J.K.M. (1984) Biochemistry 23, 508–514.
[4] Sillerud, L.O., Alger, J.R. and Shulman, R.G. (1981) J. Magn. Reson. 45, 142–150.
[5] Foxall, D.L., Cohen, J.S. and Tschudin, R.G. (1983) J. Magn. Reson. 51, 330–334.
[6] Brindle, K.M., Boyd, J., Campbell, I.D., Porteous, R. and Soffe, N. (1982) Biochem. Biophys. Res. Commun. 109, 864–871.
[7] Shaka, A.J. and Freeman, R. (1982) J. Magn. Reson. 50, 502–507.
[8] Brooks, W.M., Irving, M.G., Simpson, S.J. and Doddrell, D.M. (1984) J. Magn. Reson. 56, 521–526.
[9] Bulsing, J.M., Brooks, W.M., Field, J. and Doddrell, D.M. (1984) Chem. Phys. Lett. 104, 229–234.