Effect of strong scalar coupling in Proton-Observed Carbon-Edited NMR spectroscopy

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Introduction

^{13}C NMR spectroscopy combined with infusion of ^{13}C-labeled substrates is a powerful tool to study carbohydrate metabolism [1,2]. Indirect detection of ^{13}C label, using e.g. the Proton-Observed Carbon Edited (POCE) technique (Fig. 1), provides increased sensitivity at the expense of spectral resolution [3,4,5]. In principle, POCE yields signal only from protons bound to ^{13}C atoms, while signals from protons bound to ^{12}C are eliminated in a difference editing manner. The aim of the present study was to evaluate the effect of strong J-coupling on the editing selectivity of POCE.

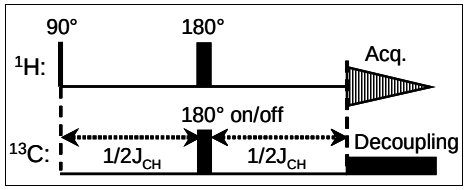

Fig.1. POCE sequence.

Methods

* *Simulations* were carried out using home-made programs in Matlab. The behavior of spin systems under the POCE pulse sequence was simulated directly using density matrix calculations with the appropriate Hamiltonians to account for evolution under RF pulses, chemical-shift and J-coupling (including strong coupling). Two different spin systems were simulated. The first spin system was a "simplified" hypothetical spin system composed of two CH groups, where one carbon was ^{13}C-labeled (i.e. ^{12}CH-^{13}CH). The parameters of this spin system were as follows: δH_1=2.34 ppm, δH_2=2.09 ppm, J_{HH}=7 Hz, J_{CH}=130 Hz. Spectra were simulated at 63 MHz with 1 Hz linewidth. The second simulated spin system was that of [3-^{13}C]glutamate. Chemical-shifts and J-coupling values were taken from Govindaraju et al. [6]. Spectra were simulated at 200, 400 and 600 MHz with 2 Hz, 3.5 Hz and 2 Hz linewidth respectively to match the linewidth of experimental data.

* *Experimental spectra* were recorded from a solution of [3-^{13}C]glutamate on three different Varian high-resolution NMR spectrometers (200, 400 and 600 MHz) under close to *in vivo* conditions (pH~7, temperature~37°C) using the pulse sequence shown in Fig. 1.

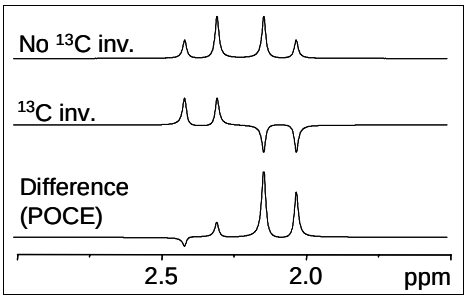

Fig.2. Simulation of POCE spectra of a hypothetical ^{12}CH-^{13}CH spin system where the two protons are strongly coupled. The signal from the ^{12}C-bound proton is not completely suppressed in the difference.

Results and Discussion

Simulation of the effect of the POCE pulse sequence (Fig. 1) on a simple strongly-coupled system ^{12}CH-^{13}CH without ^{13}C inversion pulse yielded spectra with the classical "roof effect" characteristic of strong coupling (Fig. 2, top). However, the ^{13}C inversion restored the symmetry of doublets from each proton (Fig.2, middle) similar to previously reported observations with 2D J-resolved spectra [7]. As a result, the POCE difference spectrum (Fig. 2, bottom) contained a significant residual signal from the ^{12}C-bound proton at 2.34 ppm.

Such "unwanted" signals from ^{12}C-bound protons were also observed on spectra from the more complex molecule [3-^{13}C]glutamate (Fig. 3). Simulations of POCE spectra of [3-^{13}C]glutamate (Fig. 3, third row) showed a significant signal at the GluH4 position at 2.34 ppm, well above the natural abundance level. This contamination was more prominent at lower field strength, consistent with strong-coupling effects being more pronounced at lower fields. Specifically, the area of the unwanted signal (integrated from 2.25 ppm to 2.45 ppm) was about 10% of the non-edited GluH4 resonance at 200 MHz, 6% at 400 MHz and 3% at 600 MHz. At all three field strengths, the experimental spectra of [3-^{13}C]glutamate closely matched the simulated spectra (Fig. 3, fourth row). No GluH2 signal was observed in POCE spectra at 3.75 ppm above natural abundance (not shown), confirming the absence of subtraction artifacts.

Conclusion

We conclude that POCE NMR spectroscopy does not completely suppress signals arising from ^{12}C-bound protons when strong proton-proton scalar coupling is present. This may complicate the accurate quantitation of POCE spectra.

References

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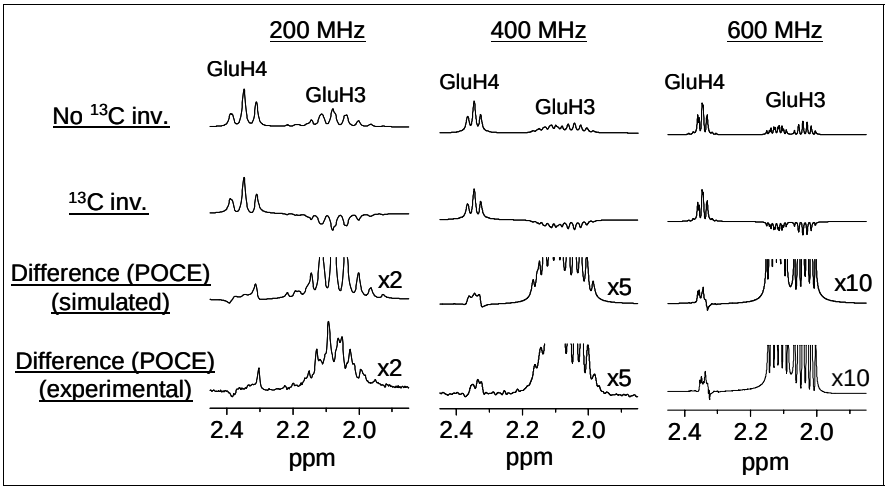

Fig.3. Simulated (top 3 rows) and experimental spectra (bottom row) from [3-^{13}C]glutamate using POCE at different field strengths. Although glutamate was not labeled at the C4 position (above natural abundance), a significant gluH4 signal was obtained at 2.34 ppm. Scaling factors indicate the scaling of edited spectra relative to unedited spectra.