Non-linear effects of strong coupling in ^{13}C edited ^{1}H NMR spectra obtained without decoupling

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Introduction

NMR measurement of metabolic fluxes by ^{13}C spectroscopy relies on the infusion of ^{13}C labeled substrate and the detection of ^{13}C incorporation into metabolites. Indirect detection methods such as the Proton-Observed Carbon-Edited (POCE) sequence [1] have been widely used to measure ^{13}C label with high sensitivity. In several recent studies [2,3,4] analysis of ^{1}H-{^{13}C} spectra has been performed using LCModel [5]. In these studies the basis-set is created from simple spectra of glutamate labeled at C3 position ([3-^{13}C]glutamate) and at C4 position ([4-^{13}C]glutamate), even though the signal of other isotopomers such as [3,4-$^{13}C_2$]glutamate is also detected. This approach is based on the assumption that [3,4-$^{13}C_2$]glutamate can be rigorously expressed as the linear combination of [3-^{13}C]glutamate and [4-^{13}C]glutamate. However, the validity of this 'linearity assumption' needs verification. The present work is the first attempt to address the assumption of the linearity of isotopomer spectra as detected by ^{1}H-{^{13}C} NMR spectroscopy. It is shown that, when ^{13}C decoupling is not performed, the linearity assumption is intrinsically incorrect for strongly coupled systems such as glutamate. On the contrary, the linearity assumption is mostly correct for the POCE sequence when ^{13}C decoupling is performed.

Theory

It can be derived from theoretical considerations on commutation of terms in the Hamiltonian (not detailed here) that, in the absence of ^{13}C decoupling, the signal evolution for a given glutamate proton (H3 for example) in [3,4-$^{13}C_2$]glutamate is not only affected by the coupling with neighboring ^{13}C nucleus C3, but also by ^{13}C nucleus C4 whose coherence is transferred *via* a two-step process implying strong homonuclear coupling (^{1}H-^{1}H or ^{13}C-^{13}C) and heteronuclear coupling. Due to this two-step mechanism, the signal of H3 in [3,4-$^{13}C_2$]glutamate is different from the signal of H3 in [3-^{13}C]glutamate. For this reason, [3,4-$^{13}C_2$]glutamate can not simply be considered as the sum of the signal of [3-^{13}C]glutamate and [4-^{13}C]glutamate. However, if ^{13}C decoupling is applied during acquisition, the two-step process described above becomes ineffective, i.e. the ^{13}C spin system has no effect on ^{1}H signal and isotopomer spectra are linear.

Methods

Numerical simulations were performed with home-made programs [6] for POCE, with echo-time minimized to $1/J_{CH}$~7.7ms, on the glutamate molecule. J_{CH} was set to 130Hz and J_{CC} was set to 35Hz. All long range heteronuclear coupling constants were considered as negligible. A 4Hz Lorentzian line-broadening was applied. Simulations were performed for [3-^{13}C]glutamate (spectrum S_3), [4-^{13}C]glutamate (S_4) and [3,4-$^{13}C_2$]glutamate (S_{34}) at 1.5T, 3T, 7T and 11.7T, without ^{13}C decoupling. In order to quantify the intensity of non-linear residual S_3+S_4-S_{34}, the residual amplitude (i.e. residual signal maximum *minus* minimum) divided by the amplitude of the doubly labeled isotopomer was calculated (R_A). The ratio of absolute value of integrals was also calculated (R_I).

Results and discussion

Non-decoupled POCE spectra for glutamate isotopomer are strongly non-linear at 1.5T (R_A=91%, R_I=20%, fig. 1A). Although spin system tends to become weakly coupled at higher fields, non-linear residuals persist (R_A=79%, R_I=15% at 3T, fig. 1B; R_A=63%, R_I=7% at 7T, fig. 1C; R_A=36%, R_I=4% at 11.7T, fig. 1D). Note that, if ^{13}C decoupling is performed, R_A and R_I are almost zero at any field (data not shown). However, even in this case, non-linear residuals do not completely vanish since the two-step process described in the theory section is still effective during the preparation period.

In practice, linear combination analysis may provide good results even with an incomplete basis-set. For example it is assessed in the context of ref. [2] (edited spectra acquired at 3T without decoupling) that LCModel is able to give a robust linear analysis of 1mM of [3,4-$^{13}C_2$]glutamate with 0.98mM of [3-^{13}C]glutamate and 1.02mM of [4-^{13}C]glutamate (not shown), despite residuals being very similar to that of fig. 1B.

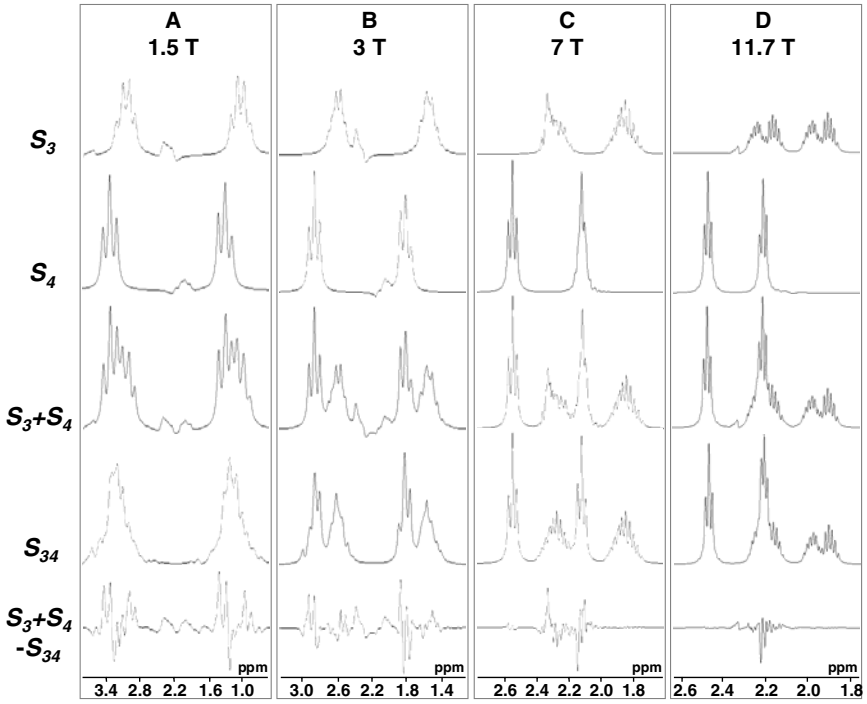

Fig. 1: POCE spectra obtained for glutamate without ^{13}C decoupling, at different B_0: A: 1.5T; B: 3T; C: 7T; D: 11.7T. Top row shows the spectrum of [3-^{13}C]glutamate (S_3), 2nd row shows the spectrum of [4-^{13}C]glutamate (S_4), 3rd row shows the sum of S_3 and S_4. 4th row shows the spectrum of [3,4-$^{13}C_2$]glutamate (S_{34}). Bottom row shows the residual S_3+S_4-S_{34}.

Perspective

In the future, non-decoupled ^{1}H-{^{13}C} NMR might become more widely used. Broadband ^{13}C decoupling becomes indeed increasingly difficult to achieve at high magnetic field. Furthermore, measuring ^{13}C label incorporation without a ^{13}C radiofrequency channel [2] precludes decoupling. Non-linearity effects will need to be to considerd in future studies performed without decoupling. Finally, analyzing non-decoupled ^{1}H-{^{13}C} spectra with an increased number of basis spectra by including multiply labeled isotopomers might make it feasible to access information on multiply labeled isotopomers using ^{1}H-{^{13}C} NMR spectroscopy.

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