Towards *in vivo* ^{13}C isotopomer analysis in the rat brain

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Carbon-13 isotopomer analysis provides unique information on relative metabolic fluxes and substrate selection. We show that ^{13}C isotopomers, previously commonly measured from tissue extracts, can also be quantified in the rat brain *in vivo* using LCModel, yielding relative concentrations for 50 different ^{13}C-labeled compounds. Even for the complex spectral pattern formed by glutamate and glutamine C2, the relative distribution of isotopomers determined *in vivo* using LCModel was in very good agreement with quantification of brain extracts.

Introduction

NMR has the unique capability of distinguishing ^{13}C label incorporation not only into different molecules, but also into different carbon positions within the same molecule (^{13}C isotopomers). The determination of the distribution of isotopomers for a given molecule (usually from tissue extracts) provides useful information on relative metabolic fluxes and substrate selection (1). However, isotopomer analysis has been challenging *in vivo* due to increased linewidth and lower signal-to-noise. The goal of the present work was to determine whether isotopomer analysis can be performed *in vivo* using LCModel (2), yielding results that are comparable to those achieved with tissue extraction.

Methods

Broadband ^{1}H-localized ^{13}C spectra were measured *in vivo* from five Sprague-Dawley rats for 2 hours on a 9.4T/31cm spectrometer using a semiadiabatic DEPT sequence (voxel 400µl) 5 h after the start of the infusion of 70%-enriched [1,6-$^{13}C_2$]glucose under α-chloralose anesthesia. Brains were funnel-frozen *in situ* and dissected under liquid nitrogen to minimize post-mortem changes, and metabolites were extracted using perchloric acid. Chemical-shifts and J-coupling values were determined from high-resolution ^{13}C spectra of brain extracts (T=37ºC, pH=7.1) acquired at 14T. The basis set for LCModel was generated by simulating each individual isotopomer with the appropriate chemical-shift and J-coupling pattern, including small effects of homonuclear ^{13}C-^{13}C J-evolution during DEPT. *In vivo* spectra were fitted with LCModel to determine the relative concentration of 50 different ^{13}C labeled compounds over a 85ppm bandwidth.

Results and Discussion

After 5 hours of glucose infusion, most metabolites exhibited a complex spectral pattern reflecting cycling of ^{13}C label into multiple carbon positions. For example, glutamate C2 appeared on extract spectra as a 9 line multiplet corresponding to the superposition of a singlet S ([2-^{13}C]glutamate), two doublets D23 and D21 ([2,3-$^{13}C^2$] and [1,2-$^{13}C_2$]glutamate) with distinct J-coupling values (J_{21}=53.4±0.1 Hz and J_{23}=34.6±0.1 Hz), and a doublet of doublets DD ([1,2,3-$^{13}C_3$]glutamate). Evidence for the presence of each of these isotopomers was clearly present *in vivo* (dashed vertical lines), even though the linewidth was broader (7 Hz) causing more overlap (Fig. 2). The fit obtained with LCModel matched closely the *in vivo* spectrum and allowed the quantification of the individual isotopomers. The *in vivo* isotopomer composition was found in very good agreement with that measured in extracts (Table). We conclude that ^{13}C isotopomer analysis is now feasible in the rat brain *in vivo* even in the case of complex spectral patterns with significant overlap, allowing a complexity of spectral analysis hitherto unachievable *in vivo*.

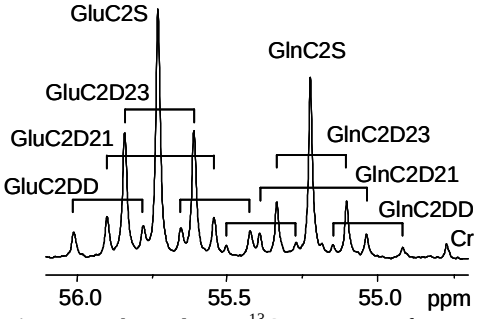

Fig. 1. High-resolution ^{13}C spectrum of a rat brain extract (14 Tesla) showing the complex spectral pattern of glutamate and glutamine C2 resonances after [1,6-$^{13}C_2$]glucose infusion. Isotopomer nomenclature similar to (1).

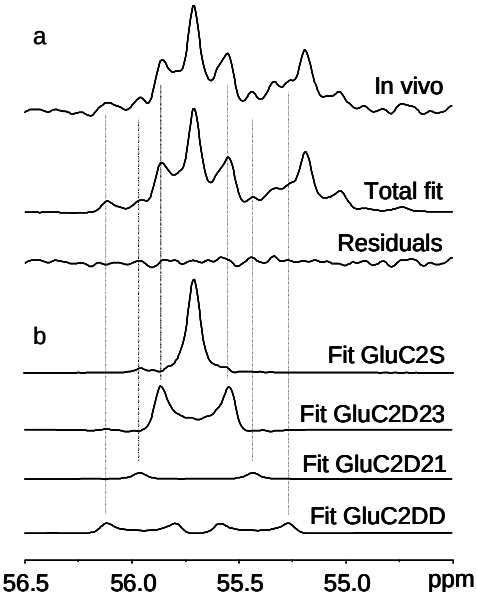

Fig. 2. (a) In vivo ^{13}C spectrum (9.4T, 2 hours acq.) and LCModel fit of glutamate and glutamine C2. (b) contribution of each glutamate isotopomer to the fit.

	In vivo	extract
gluC2S	(41±1)%	(36±2)%
gluC2D23	(40±4)%	(37±1)%
gluC2D21	(4±2)%	(12±1)%
gluC2DD	(14±7)%	(15±1)%

Table. Comparison of isotopomer distribution for glutamate C2 determined from *in vivo* and extract data in the same animal.

References

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