Automatic quantitation of *in vivo* ^{13}C spectra using LCModel

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Our goal was to demonstrate that LCModel, which was originally designed for ^{1}H MRS, can also be used to quantify *in vivo* ^{13}C NMR spectra. Using this approach, we analyzed localized ^{13}C spectra obtained at 9.4T in the rat brain during ^{13}C-labeled glucose infusion. The fully automatic and user-independent analysis resulted in the simultaneous quantitation of *in vivo* ^{13}C labeling time courses for more than ten singlets (including glucose C1, glutamate C2,C3,C4, glutamine C2,C3,C4, aspartate C2,C3, and NAA C6) as well as eight ^{13}C-^{13}C isotopomers. This provides an unprecedented amount of information for metabolic modeling.

Introduction

In vivo ^{1}H-localized ^{13}C spectroscopy in the rat brain at 9.4T allows for the simultaneous detection of the entire carbon spectrum as well ^{13}C-^{13}C isotopomers, providing a wealth of information. Traditionally, the measurement of direct-detected ^{13}C NMR spectra is done manually by integration or simple peak fitting, a time-consuming and user-dependent procedure. Incorporation of more prior knowledge should improve and simplify quantification. The goal of this work was to test if LCModel (1), originally designed to be used on ^{1}H spectra of the brain, can be used to analyze ^{13}C NMR spectra.

Methods

Experiments were carried out on a 9.4T magnet (Magnex/Varian). The surface probe consisted of a ^{13}C linear coil and a ^{1}H quadrature coil. ^{13}C spectra were acquired using a ^{1}H-localized polarization transfer sequence (2). Male Sprague-Dawley rats were infused with 70% [1-^{13}C]glucose and spectra were collected in a 400µl volume with a temporal resolution of ~5 minutes (128 scans, TR 2.5s) over a glucose infusion period of 4 hours. Each 5 min ^{13}C spectrum was analyzed using LCModel.

For LCModel analysis, the basis set of model spectra was generated by simulating Lorentzian lines at adequate chemical shifts based on published values that were verified experimentally. ^{13}C-^{13}C doubly labeled isotopomers were incorporated in the basis set as doublets. Undetectable isotopomers were not included in the basis set. Data were preprocessed to allow proper referencing by LCModel, which normally accepts only ^{1}H spectra. Time series of ^{13}C spectra were analyzed fully automatically without any user intervention. Correction factors were applied to account for differences in polarization transfer efficiency (eg CH vs CH_2 groups) and off-resonance effects.

Results and Discussion

The entire *in vivo* ^{13}C spectrum was fitted in a single analysis (Fig.1), spanning from 100 ppm (β-glucose C1 at 96.7 ppm) to 20 ppm (NAA C6 at 22.9 ppm) and including ^{13}C-^{13}C isotopomers (Fig.2). The quality of the fit was confirmed by the flat residuals.

When analyzing 6 consecutive spectra at isotopic steady-state (i.e., at the end of the time course), the experimentally measured coefficient of variation (CV) was consistent with the Cramer-Rao bounds (CRB) provided by LCModel (shown for glutamate in Table 1). Fitting the sum of these 6 spectra (32 min acq) resulted in the expected improvement in signal-to-noise, as judged from the CRB.

Figure 3 shows the time courses of glutamate and glutamine C4 and C3 from a single animal. Total ^{13}C concentrations were obtained by adding isotopomers for a given metabolite after LCModel analysis (e.g. total glutamate C4 = Glu_4+Glu_{43}).

We conclude that LCModel can be used to analyze *in vivo* ^{13}C spectra time series in a robust and fully automatic manner. This will be useful for modeling the complex compartimentalized metabolism of the brain (3,4).

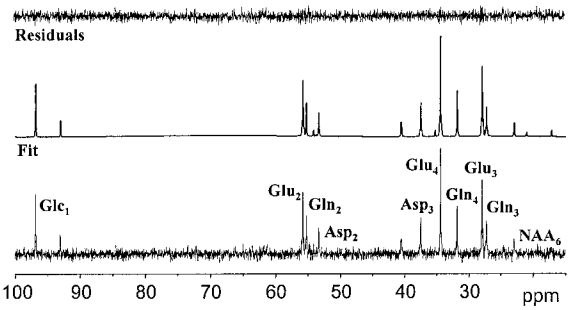

Fig.1 LCModel fit of an *in vivo* ^{13}C spectrum (400 µl, 32 min).

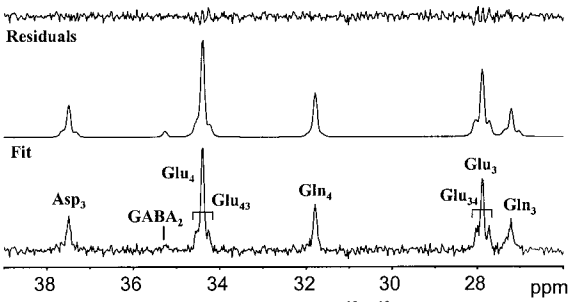

Fig.2 Expansion of Fig.1 showing the fit of ^{13}C-^{13}C isotopomers.

Metabolite	32 min spectrum		6 x 5.4 minutes spectra		
	Conc. (mM)	CRB (%)	Mean Conc. (mM)	CV (%)	Mean CRB (%)
Glu_4	1.99	2	2.08	4	5
Glu_{43}	0.41	11	0.32	39	43
Glu_3	1.48	3	1.55	5	6
Glu_{34}+Glu_{32}	0.63	8	0.55	17	24

Table 1 Resulting fitted ^{13}C concentrations for glutamate C4 and C3. (CRB=Cramer-Rao Bound, CV=Coefficient of Variation).

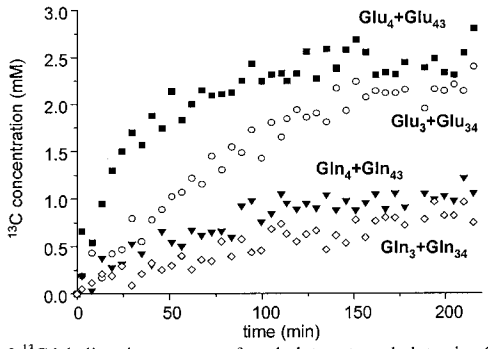

Fig. 3 ^{13}C labeling time courses of total glutamate and glutamine C4 and C3 in a single animal analyzed automatically using LCModel.

References

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