Spectral Fitting of High Resolution Rat Brain Extract NMR Data by LCModel with a Simulated Basis Set

A. Borgert[1,2], K. O. Lim[1,2], and P-G. Henry[1,3]
[1]Center for Magnetic Resonance Research, University of Minnesota, Minneapolis, MN, United States, [2]Department of Psychiatry, University of Minnesota, Minneapolis, MN, United States, [3]Department of Radiology, University of Minnesota, Minneapolis, MN, United States

Introduction

Spectra from brain extracts contain a wealth of metabolic information that cannot always be measured *in vivo* due to limitations in sensitivity (eg: metabolites with low concentration, small region of interest) and/or spectral resolution. Spectral fitting methods such as commercial metabolomics software (eg, Chenomx[1]) or capabilities built into NMR system software (eg, Varian or Bruker) require significant user input and are generally not amenable to automation, making them time-consuming, cumbersome, and prone to user error. To address these issues, we have adapted the LCModel [2] software package for use with high resolution *in vitro* NMR data, allowing for automated and consistent analysis of such data.

Methods

Rats were anesthetized with 5% isoflurane and euthanized via microwave fixation. The brain was removed and processed using a standard PCA extract protocol. Samples were lyophilized, redissolved in 99.9% D2O and the pH adjusted to 7.0 +/- 0.1. NMR spectra were acquired using a Bruker 16.4T spectrometer running under full automation using a pulse-acquire sequence with residual water presaturation and TR = 10s. Sample temperature was maintained at 37° C. The LCModel basis set was generated via density matrix simulation in MATLAB [3], with separate basis spectra generated for majority of individual metabolite protons. The fit region ranged from 0.2 to 4.2 ppm with gaps at the DSS resonances (0.6, 1.75 and 2.9 ppm) and from 3.3 to 3.8 ppm to compensate for the presence of significant unassigned contaminates.

Results and Discussion

Figure 1 shows part of an example fit generated with this method. In general, the fit appears quite good, with the only significant residuals resulting from minor unassigned resonances around 3.25, 2.52 and 2.01 ppm. GABA, NAA, creatine, aspartate and the H4 resonances of glutamine and glutamate can be consistently quantified with CRLBs of less than 5%, while low concentration metabolites and those with significant overlap were consistently quantified with CRLB < 10%. Fit quality in the region of 3.8 to 4.2 ppm (not shown) was of generally lower quality, although creatine, phosphocreatine and inositol could be consistently quantified with CRLB < 10%. Due to the narrow linewidth and high resolution of modern high-field MRS systems, precise control of sample temperature and pH is generally required to prevent discordant changes in the chemical shifts of protons within same metabolite. While temperature control is generally a simple matter *in vitro*, the time and effort required for proper pH control is not trivial. By using a simulated basis set and allowing the chemical shifts of protons within the same metabolite to vary independently, simple coarse adjustment of the sample pH (within +/- 0.2 of the target pH) is generally sufficient to ensure robust fitting across multiple samples.

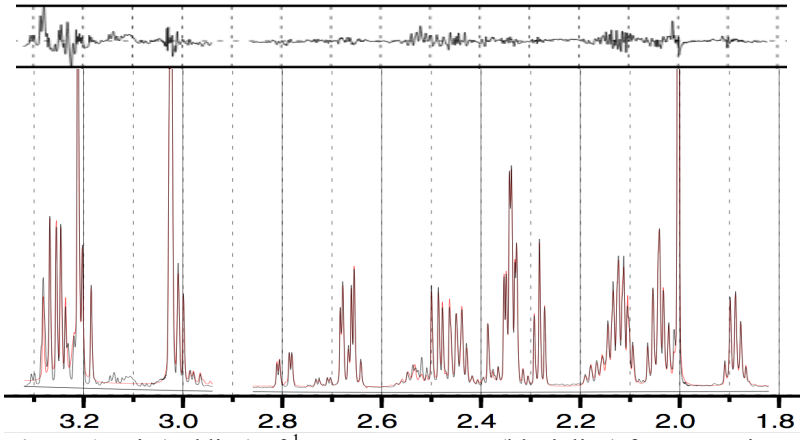

Figure 1: Fit (red line) of ^{1}H NMR spectrum (black line) from rat striatum. The fit residual is shown at the top of the figure.

Conclusions

Widely used to fit *in vivo* MRS data, LCModel is equally capable of fitting high resolution *in vitro* MRS data as well. The automated nature of the LCModel fitting procedure, combined with a simulated basis set, significantly reduces the need for direct user input and thus user error and inter-user variation in the resulting concentration values.

Acknowledgements

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References

[1] Chenomx Inc., Edmonton, Alberta, Canada; [2] Stephan Provencher Inc., Oakwood, CA, USA; [3] Henry et al. MRM 2006;