Natural Abundance Applications to ^{13}C Metabolomics

B. Lee-McMullen, C. Clendinen, G.S. Stupp, K. Vandenborne, G. Walter, A.S. Edison (University of Florida)

Introduction

High temperature-superconducting (HTS) probe[1] optimized for ^{13}C, has been shown to have higher ^{13}C mass sensitivity and to decrease the time to acquire a natural abundance carbon spectrum. Due to the narrow peak widths and the larger spectral width, we hypothesized that carbon spectra will improve interpretation of multivariate statistical analysis from proton spectroscopy alone.

Experimental

We use a synthetic data, comprised of two groups of amino acid mixtures, to expand established methods for data mining and identification of metabolites to incorporate ^{13}C data. This approach was applied to serum from the Duchenne muscular dystrophy mouse model (*mdx*) and controls. From both the synthetic mixture and the serum, 1D ^{1}H and ^{13}C spectra were acquired using an Agilent 600 MHz high-resolution NMR equipped with a custom HTS probe shown to have two times more mass sensitivity than commercially available probes. Initial multivariate analysis was performed on ^{1}H and ^{13}C spectra separately. We were then able to perform a statistical heterospectroscopy[2], from 1D ^{1}H and ^{13}C spectra to interpret proton-carbon interactions.

Results and Discussion

Synthetic mixtures were used to apply statistical analysis of the 1D spectra to optimize metabolic profiling with the addition of ^{13}C spectra. When applied to the mouse serum we found that 1) carbon spectra separated the two groups better and 2) correlation between carbon and proton reveals a more complete metabolic profile. Using partial least squares discriminant analysis, we compared carbon and proton Q^2 scores, where a score closer to one represents a better model. We found that ^{13}C serum spectra had a higher Q^2 scores than that of proton (0.67 vs 0.21). We also found that we are able to compute metabolite profiles using statistical heterospectroscopy that would be impossible solely on the proton spectra (Figure 1).

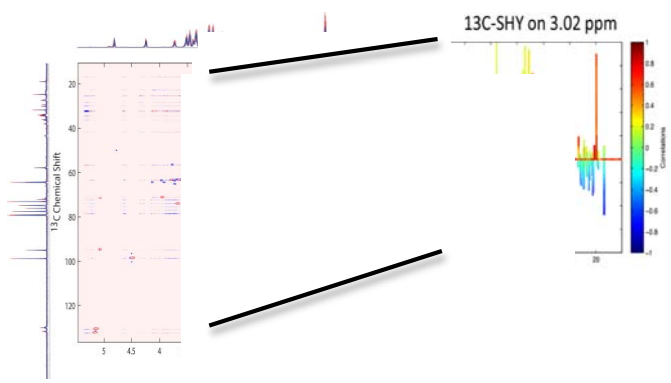

Figure 1.
The 2D ^{13}C-Statistical heterospectroscopy plot shows full resolution ^{13}C covariance with the ^{1}H resonances. The individual ^{1}H resonance at 3.02 ppm was separated to show the correlated carbons one dimensionally.

Conclusions

Using innovative HTS probes, ^{13}C sensitivity was increased allowing for the acquisition of both ^{1}H and ^{13}C spectra. Combining ^{13}C/^{1}H analysis with established multivariate statistics allowed for the determination of a more comprehensive metabolic profile in mouse sera than using proton spectroscopy alone.

Acknowledgements

We would like to acknowledge our funding sources including the “Magnetic Resonance Imaging and Biomarkers for Muscular Dystrophy” (NIH/NIAMS RO1) , National High Magnetic Field Laboratory, and the TL1 training grant from the CTSI.

References

[1] Ramaswamy, V.; Hooker, J. W.; Withers, R. S.; Nast, R. E.; Brey, W. W.; Edison, A. S. *Journal of Magnetic Resonance* **2013**.
[2] Crockford, D. J.; Holmes, E.; Lindon, J. C.; Plumb, R. S.; Zirah, S.; Bruce, S. J.; Rainville, P.; Stumpf, C. L.; Nicholson, J. K. *Analytical chemistry* **2006**, *78*, 363.