^{13}C-Enriched Global Metabolomics Approach Using 2D ^{13}C NMR

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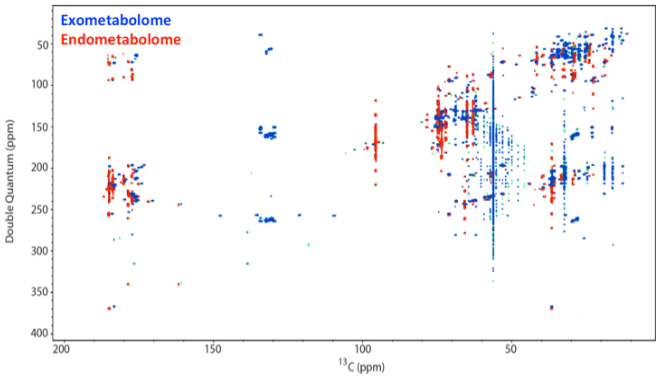
Introduction

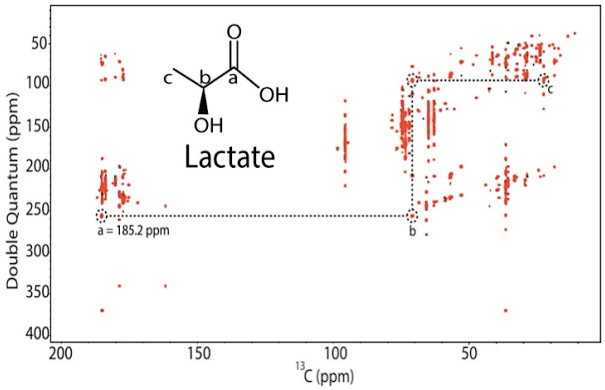
Typically in a global metabolomics study, metabolic mixtures are collected and characterized by ^{1}H NMR and/or LC-MS. Usually with NMR-based metabolomics, 1D and 2D ^{1}H-detected methods are used to analyze the global response of a biological system to perturbation/stress. Though ^{1}H-detected methods are extremely useful, 1D ^{1}H NMR of mixtures can suffer from heavy peak overlap due to low spectral dispersion (~12 ppm) as well as problems with signals that arise from solvents such as water. The limited use of ^{13}C-detected methods stems from the difficulty of acquiring spectra; this is due to the low sensitivity of ^{13}C at natural abundance. 2D techniques such as ^{13}C-^{13}C 2D-INADEQUATE mixture analyses are made possible with enrichment. In conjunction with well-established statistical techniques such as principal component analysis (PCA), we are developing a method for analyzing 2D-INADEQUATE data using the ^{13}C-enriched exo- and endometabolomes of *C. elegans* under stressful conditions.

Experimental

Our lab developed a technique to successfully isotopically ^{13}C label *C. elegans* with ^{13}C. We tested *C. elegans* metabolic response to heat shock by incubating a ^{13}C labeled population in a warm water bath. Both endo and exometabolites were collected. Both were concentrated and placed in 1.5mm tubes and using the 1.5mm Varian cold probe at the Amris facility at the University of Florida, we collected INADEQUATE spectra of both the endo and exometabolites. Exometabolome data were collected with a 3 second relaxation delay, 512 t1 increments, 0.134s acquisition time, and 16 scans. This experiment took approximately 11 hours. Due to the presence of T1 ridges, we reduced the number of scans per increment to 4 and increase t1 to 2048 increments.

Results and Discussion





The figure on the left shows an overlay of the endo and exometabolome. Increasing the number of increments greatly reduced the presence of the T1 ridges. Higher order coupling was observed in these labeled samples. The figure on the right displays one of the compounds that have thus far been identified using this method in the endometabolome.

Conclusions

We were able to successfully label *C. elegans* and collect INADEQUATE spectra of *C. elegans* endo and exometabolites. As shown above, we can see clear differences between the endo and exometabolome particularly in the sugar regions and chemical shifts indicative of ribonucleotides exist. We are currently working on automated means of metabolite identification in INADEQUATE spectra of mixtures.

Acknowledgements

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References

[1] Ramaswamy,V. *et al.* eMagRes. 2013