A Natural Abundance ^{13}C NMR Approach for Metabolomic Studies of Complex Mixtures

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Introduction

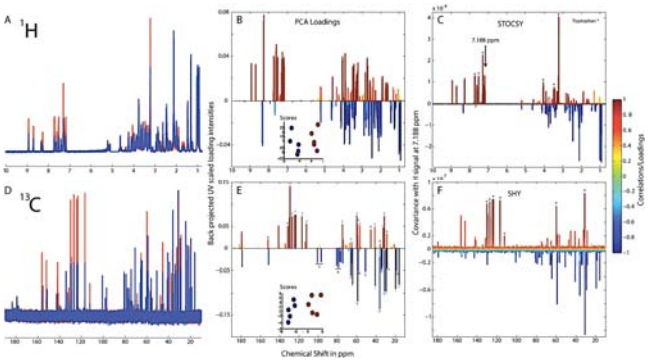
Typically in a global metabolomics study, metabolic mixtures are collected and characterized by ^{1}H NMR and/or LC-MS. Usually with NMR-based metabolomics, 1D and 2D ^{1}H-detected methods are used to analyze the global response of a biological system to perturbation/stress. Though ^{1}H-detected methods 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 advantages of ^{13}C NMR techniques over traditional ^{1}H NMR include the carbon spectral width, which is much larger (>200 ppm) and therefor affords greater spectral dispersion. Here, we use ^{13}C NMR to potential of ^{13}C-detected NMR metabolomics at natural abundance. In order to develop and validate the approach, we first show results from synthetic mixtures of 20 common metabolites. We then applied the overall approach to mice serum and *D. melanogaster* extracts.

Experimental

One-dimensional ^{1}H and ^{13}C spectra were collected on an Agilent VNMRS-600 spectrometer using our custom 1.5-mm ^{13}C HTS probe (Ramaswamy, 2013). Synthetic mixture and fly extract ^{1}H data were collected with a simple pulse sequence with preset of residual water with a spectral width of 12 ppm (7183.9 Hz) at an observe frequency of 599.68 MHz. Synthetic mixture and fly ^{13}C spectra were collected on a total time of 2hr 3min, with a 212 ppm (32051.3 MHz) with an observe frequency of 150.79 MHz; 0.10 sec relax delay; 0.800 acquisition time. Both used a 60-degree pulse. All ^{1}H was decoupled at 599.68 MHZ with a power of 37 dB continuously on WALTZ-16

Results and Discussion

^{13}C NMR spectra were collected for each sample and are shown in Figure 1A and 1D, respectively. Inspection of the NMR spectra clearly indicates the increased spectral dispersion of the ^{13}C (Fig 1D) compared to the ^{1}H data (Fig 1A) from the same sample. PCA was performed on each set of data, and the results are shown in Figures 1B (^{1}H) and 1E (^{13}C). The scores plots are shown as an inset in the loadings plots from each set of data. PCA was sufficient to separate both the ^{1}H and ^{13}C datasets along PC1, but it is clear from the loadings that the ^{13}C results (Fig 1E) are simpler to interpret because of considerably less overlap than the ^{1}H loadings (Fig 1B). Figure 1C shows STOCSY results from the ^{1}H data (Fig 1A). The Trp 7.19 ppm resonance was used as the driver peak, and not surprisingly other Trp peaks were strongly correlated.



Conclusions

The HTS probe allows for minimized time and sample allowing for carbon metabolomics. The synthetic mixtures provided proof of concept that we would be able to identify metabolites within 40-200 nM. As a proof of concept, fly mixtures were put analyzed using this workflow. Unlike the ^{1}H spectra, the ^{13}C spectra were easily annotated. The 1H spectra contained heavy overlapped region that made annotation very difficult with the exception of peaks that were not overlapped.

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References

[1] Ramaswamy,V., *et al*., eMagRes, **2**, 215-228 (2013).