SMALL VOLUME METABOLOMICS OF RODENTS

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Introduction

Despite recent advances in molecular biology that have provided a fuller understanding of the transcriptional basis of disease, there is a paucity of technology that allows the ultimate end product of transcription, metabolic flux, to be measured in vivo. The importance of measuring flux is highlighted by a number of examples in animal models where changes in enzyme expression do not match changes in flux through the enzyme. Thus, if the metabolic basis of disease is to be explicitly understood, techniques that measure in vivo fluxes must be further developed so that they can be easily accessed in basic and clinical science. NMR isotopomer analysis of small molecule metabolites is well suited to make these measurements because chemical shift and spin-spin coupling allow the isotopomer populations to be very well defined. Knowledge of isotopic distributions in metabolites is tantamount to knowing the flux through the biochemical pathways that generated the isotopomers.

Experimental

A mouse heart was perfused with [U-^{13}C] free fatty acids and [1,6-^{13}C]glucose. With this labeling scheme, the C4 position of glutamate is the most informative. The doublet of doublets represents free fatty acid oxidation while the doublet due to C3-C4 coupling represents glucose oxidation. We dissolved the lyophilized sample (50 ug, 50% ^{13}C enriched) in 100 mL D_2O and then utilized 8 μL of that sample for NMR analysis at 600 MHz using the ARMIS/NHMFL 1-mm HTS probe. We collected high-quality spectra with good S/N: ^{1}H 1D (32 scans), ^{13}C-1D (~10 h), ^{13}C-HMQC (~20 m), and ^{13}C-JHSQC (~2 h). The JHSQC data (Figure 1) are particularly useful for high-sensitivity ^{1}H-based detection of ^{13}C multiplet structure (1), and this is an ideal application for the 1-mm HTS probe that is optimized for ^{1}H detection.

Results and Discussion

We were able to obtain good S/N for the small sample volume (Fig 1). This demonstrates that small volumes of sample can be successfully measured using the 1-mm HTS probe.

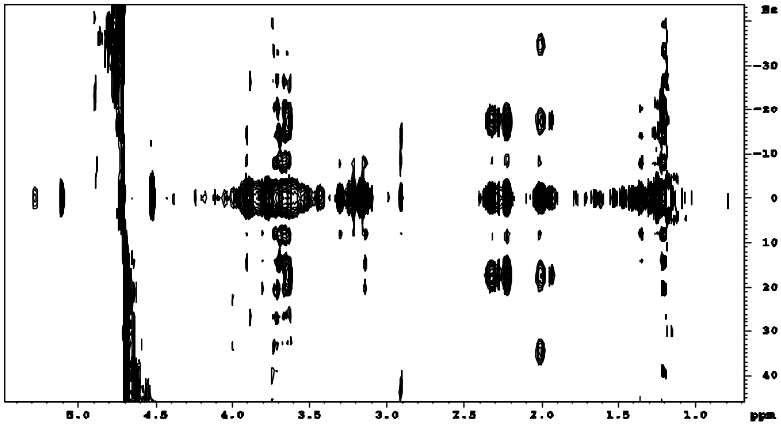

Figure 1) ^{1}H-^{13}C-JHSQC spectrum of a mouse heart perfused with [U-13C] free fatty acids and [1,6-13C]glucose. Data were collected with 64 t_1 points and 48 scans at 600 MHz using the 1-mm HTS probe. The sample was prepared as described in the text.

Conclusions

The 1-mm triple resonance HTS probe has extremely high 1H mass sensitivity and thus is an ideal technology to investigate patterns of ^{13}C distribution indirectly through their attached ^{1}H, techniques pioneered by the UTSW group. This could allow time-course studies to be done efficiently and with modest cost in materials and animals.

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References

[1] Burgess, S. C., et al., Analytical Biochemistry **289**, 187-195 (2001).