Nuclear Magnetic Resonance (NMR) Spectroscopy

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1D NMR

Blood Based Metabolomics

Metabolites

CPMG

Protein + Metabolites

NOESYPR
Limitations of Intact Blood serum/plasma Analysis

- Far fewer number of metabolites ~ 30 or less
- Suppression or attenuation of metabolite peaks due to protein binding

Alternative Blood Analysis Methods

- Precipitation
- Ultrafiltration
Comparison of Blood Metabolite Analysis Methods

**Precipitation**

- Formate
- 3-Methylhistidine
- Histidine
- Tyrosine
- 1-Methylhistidine
- Phenylalanine
- Tryptophan
- Uridine
- Benzoate

**Ultrafiltration**

**CPMG**

Suppression: Physically removing large molecules

Metabolites Extraction using protein precipitation:

- Add methanol 2:1
- Precipitate protein
- Centrifuge, remove protein precipitate
- Dry the supernatant and reconstitute in water

Filtration using molecular weight cut-off filters (3kDa)

Extracted samples can be used for both MS and NMR analyses


Anal Chem. 2015 Jan 6;87(1):706-15
Identification and Quantitation of Blood Metabolites

- Nearly 70 metabolites identified and quantitated in human blood plasma

Whole Blood Metabolomics using NMR

- Nearly 80 metabolites identified and quantitated in whole human blood

Anal Chem. 2015 Jan 6;87(1):706-15

Anal Chem. 2017 Apr 18;89(8):4620-4627
Blood metabolites extraction: Choice of solvent

Concentration (uM)

Acetonitrile not good for protein precipitation

Anal Chem. 2015 Jan 6;87(1):706-15

Coenzymes and Antioxidants
Analysis in Tissue in one step

Many coenzymes are unstable
Challenging for MS

Simultaneous Analysis of Coenzymes

Mice hearts
n=6

Sensitivity to Harvesting/Extraction

Mouse Heart

MeOH-Water

MeOH-CHCl₃, in vivo

MeOH-CHCl₃, perfusion

MeOH-CHCl₃
Urine NMR: Sample Preparation

Urine - Intact samples
No preparation required except adding buffer to stabilize pH
About 0.1% NaN₃ added to prevent bacterial growth

More number of metabolites detected by NMR than MS

The Human Urine Metabolome

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Abstract

Urine has long been a “forgotten” biofluid among metabolomic researchers. It is sterile, easy-to-obtain in large volumes, largely free from interfering proteins or lipids and chemically complex. However, this chemical complexity has also made urine a particularly difficult substrate to fully understand. As a biological waste material, urine typically contains metabolic breakdown products from a wide range of foods, drugs, environmental contaminants, endogenous waste metabolites and bacterial by-products. Many of these compounds are poorly characterized and poorly understood. In an effort to improve our understanding of this biofluid we have undertaken a comprehensive, quantitative, metabolome-wide characterization of human urine. This involved both computer-aided literature mining and comprehensive, quantitative experimental assessment/validation. The experimental portion employed NMR spectroscopy, gas chromatography mass spectrometry (GC-MS), direct flow injection mass spectrometry (DFI-MS/MS), inductively coupled plasma mass spectrometry (ICP-MS) and high performance liquid chromatography (HPLC) experiments performed on multiple human urine samples. This multi-platform metabolomic analysis allowed us to identify 445 and quantify 378 unique urine metabolites or metabolite species. The different analytical platforms were able to identify (quantify) a total of 209 (20%) by NMR, 179 (83) by GC-MS, 127 (127) by DFI-MS/MS, 40 (40) by ICP-MS and 10 (10) by HPLC. Our use of multiple metabolomic platforms and technologies allowed us to identify several previously unknown urine metabolites and to substantially enhance the level of metabolome coverage. It also allowed us to critically assess the relative strengths and weaknesses of different platforms or technologies. The literature review led to the identification and annotation of another 226 sensory compounds and was used to help guide the subsequent experimental studies. An online database containing the complete set of 2651 confirmed human urine metabolite species, their structures (3079 in total), concentrations, related literature references and links to their known disease associations are freely available at http://www.urinemetabolome.ca.
Preparation of Cells and Tissue for NMR

Magic angle spinning for intact cells and tissue samples

54.7° Magic Angle

Nuclei interact with other nuclei within the molecule and between molecules

Short Chain Fatty Acids Analysis using 1H NMR

- Formate
- Acetate
- Butyrate
- Propionate
- Succinate
- Fumarate
- Pentatonic acid
- Hexanoic acid
Metabolite identification in salty samples (3% salt)

$^1$H NMR at 800 MHz

- Formate
- Beta-hydroxybutyrate
- Glycerol
- Acetate
- Lactate

Distinguishing Isomeric Compounds: $^{13}$C NMR

Conjugated Linoleic acid (CLA)
NMR of isolated organs

Mouse heart

$^{31}$P NMR

J Vis Exp. 2010; (42): 2069.

NMR of rodents
NMR of Animals

NMR of Humans

$^1$H NMR

$^{31}$P NMR

Imaging

Angiography

Functional Imaging
**Sample Preparation**

Thaw frozen sample

1. Intact sample - Use directly for NMR
2. Processed sample - ultrafiltration, protein precipitation (drying)
3. Mix/dissolve with D$_2$O solvent/buffer (TSP/DSS)
4. Run NMR

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**NMR Data Pre-Processing**

Raw data: FID (free induction decay)

\[ ^{1}H \text{ NMR} \]
\[ ^{13}C \text{ NMR} \]
\[ ^{31}P \text{ NMR} \]
\[ \text{etc.} \]
Apply window function
Smoothening or resolution enhancement

- exponential
- gaussian
- sine
- squared sine
- etc

FT (Fourier transformation)
Phase correction: After

Baseline correction: Before
Baseline correction: After

Calibration (peak alignment): Before
calibration (peak alignment): After

Binning
Peak integration – relative quantitation

Absolute quantitation