Future Directions: Tissue and Cell Imaging
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Outline:
A. Brief introduction of tissue molecular imaging by mass spectrometry
B. Sample preparation issues: tissue preparation, matrix application
C. Compound identification: Molecular weight (high resolution) and MS/MS
D. Brain images-Abundance of Ions
   i. Abundance of ions/abundance of phospholipids
   ii. Microdissection and LC/MS/MS quantitation
E. Buckyball \((C_{60}^+)\) images
F. Future
G. References
Matrix: 2,5-dihydroxybenzoic acid

N₂-laser
337 nm

Matrix: 2,5-dihydroxybenzoic acid
MALDI Imaging of Lipids

(Richard Caprioli-protein/peptides)
Sublimation

- Tissue (Dried)
- Dry ice acetone
  -20°
- Sublimation
- 2,5-Dihydroxy Benzoic acid (matrix)
- Vacuum
Sample preparation

- Mouse brain flash frozen-70°C
- Warmed to -15°C, mounted with Optimal cutting temperature compound
- Sliced (cyrostat) 10 um thickness
- Placed directly on glass cover slips or MALDI steel plate
- Stored -20°C
Positive ions

16:0a/18:1-PC
m/z 760.6
Full Scan Spectrum of image

- m/z 760.6
- m/z 798.6
- m/z 826.6
- m/z 734.6
- m/z 651.5
- m/z 577.5

Signal

m/z

500 600 700 800 900 1000 1100
Identification of Lipids (MS/MS)

Cerebral Cortex
Corpus Callosum
Thalamus
Striatum
Hippocampus
Cerebellum
Medulla
Pons
Hypothalamus

m/z 760.6

MS/MS

Relative Intensity

m/z 100 300 500 700 900

m/z 184.1 760.6
Docosahexaenoic Acid Containing Phosphatidylcholine

18:0α/22:6-PC
m/z 834.6
Negative ions

d18:0/24:1 - ST
m/z 888.7

18:0α/20:4-PI
m/z 885.6

Stain image
Allen Brain Atlas
www.brainatlas.org
Negative Ions

18:0a/22:6-PS
m/z 834.6

Cerebellum

MS/MS(CID)
m/z 834.6
What do the ion abundances mean?

- Phospholipid species is present?
- Concentration is higher than other regions?
Is there more 16:0/16:0-PC (m/z 734.4) in cortex than in corpus callosum?
Is there really more esterified 22:6 in the rat cerebellar grey matter?
Is there really more esterified 18:0/18:1 in the rat white matter?
Do observed ions reflect actual concentrations?

- Image of major phospholipids from a slice
- Isolate mouse brain regions (micro dissection) on adjacent slice
- Extraction (added 100ng deuterated-PC internal standard)
  - Normal phase LC/MS (electrospray ionization)
    - Positive ions
Abundance of ions in images:
False negatives/no false positives

• Imaging of Phospholipids in tissue slices
  - Striking distribution of specific molecular species in regions (50 micron resolution)
  - Observance of m/z means PL is present at site
    • False negative information likely
  - Mechanism of lipid secondary ion release?
• Abundance of ions
  • \( I_{m/z} = f([\text{Lipid}] \times [\text{ionization cross section}] \times [\text{local environment}] \times ...) \)
Why is local environment so different about white matter?

Myelin sheath
Total MALDI ions in celebellar grey (blue) and celebellar white (red)
Collaboration:
Nick Winograd Penn State

- SIMS (secondary ion mass spectrometry) based imaging
  - Buckyball ion beam ($C_{60}^+$)
  - Better lateral resolution
    - Lipid bilayers with SIMS ($Ga^+$) Science, 2004
    - 200 nm ion beam

- Compare sublimation MALDI with SIMS
  - Prepare rat cerebellum on In oxide glass slides
  - Serial sections analyzed UCHSC/Penn State
Buckyballs $[C_{60}]^+$

Nick Winograd-Penn State

Primary ion beam focused to a submicron spot

Each carbon atom carries $\frac{1}{60}$th of total incident kinetic energy

Other ion beams - $Au_3$, $Bi_3$, $SF_9$, $Au_{400}$

$C_{60}$ Ion Source

Spatial Resolution $< 300$ nm
Energy range $10$ keV - $40$ keV
Source lifetime $> 600$ hours
Cholesterol m.z 369
PC head group m/z 184
Conclusions

• Imaging of Lipids in the brain
  – Striking distribution of specific PL-molecular species and even cationized species
  – Abundance information relevant within tissues of similar cellular structure
  – Rich biochemical information
  – Can we improve lateral resolution?
G. Examples of imaging lipids in tissues


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