Strategies for Optimizing Detection of Endogenous Metabolites Directly from Tissue via MALDI IMS

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OVERVIEW

Purpose

- To examine whether heat stabilization and protease inhibitors can improve the detection of small endogenous metabolites from tissue sections via MALDI IMS

Methods

- Rodent brain tissues were either fresh frozen or heat stabilized (Stabilizor, Denator)
- Plates were pre-coated with CHCA with or without protease inhibitors
- 12 µm thick tissue sections were thaw-mounted onto pre-coated MALDI target plates
- Plates were post-coated with 9-aminoacridine (9AA)
- Data were obtained in negative ion mode:
  - with a Thermo LTQ XL mass spectrometer (MS^3)
  - with a Bruker 9.4 T solariX FTICR mass spectrometer (accurate mass)

Results

- Both heat stabilization and phosphatase inhibitors resulted in higher ATP levels and lower ADP levels compared to fresh frozen brain tissues
- ATP/ADP was increased ~2x with phosphatase inhibition alone and ~2.5x with heat stabilization

INTRODUCTION

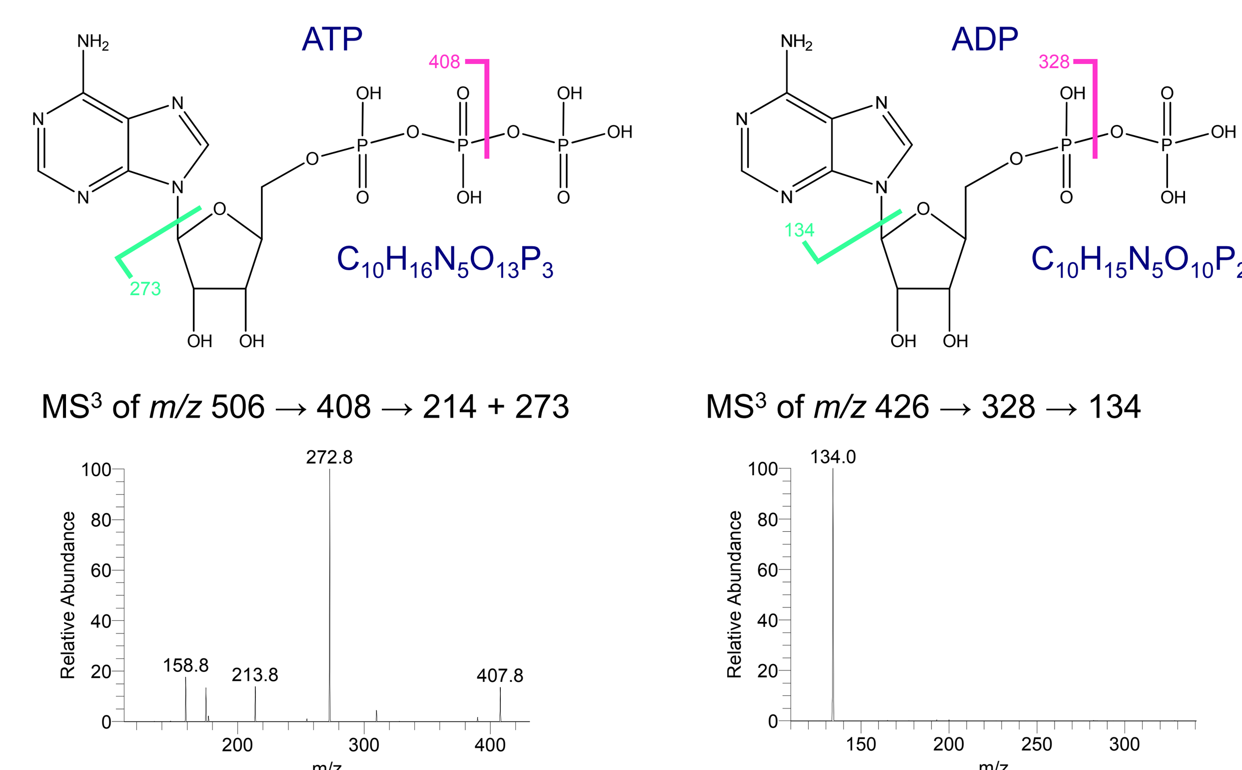
The study of small molecule metabolites in biological systems can impart a great deal of knowledge about a given organism. MALDI imaging mass spectrometry (IMS) is uniquely able to provide the spatial localization of many different analytes, including endogenous small molecules.[1] However, many endogenous metabolites are quickly degraded post-mortem. For example, it is well known that ATP rapidly degrades in the brain after death.[2] We present here strategies for optimizing the spatial detection of endogenous metabolites in tissue by minimizing enzymatic activity through the use of protease inhibitors and heat stabilization.[3]

METHODS

Brains from control rats were used. Animals were sacrificed, and the brains were dissected and cut in half. Half of each brain was snap frozen, and the other half was heat stabilized using the structure preserve setting on the Stabilizor T1 instrument (Denator). After freezing or stabilization, the tissues were stored at -80°C until further analysis. Tissues were sectioned into 12 µm thick slices and thaw-mounted onto MALDI target plates precoated with CHCA (5 mg/ml, 90:10 acetonitrile:water) using a TM Sprayer with or without protease inhibitor (SigmaFAST protease inhibitor tablet). The tissues were then post-coated with a solution of 9-aminoacridine (9AA, 5 mg/ml, 90:10 methanol:water) using a TM Sprayer. Targeted analyses for ATP and ADP were performed in negative ion mode on a linear ion trap instrument equipped with a MALDI source (LTQ XL, ThermoFisher) utilizing optimized MS^3 conditions. Images were acquired in spiral raster mode, with 200 µm raster and 100 µm spiral raster resolution on the LTQ.

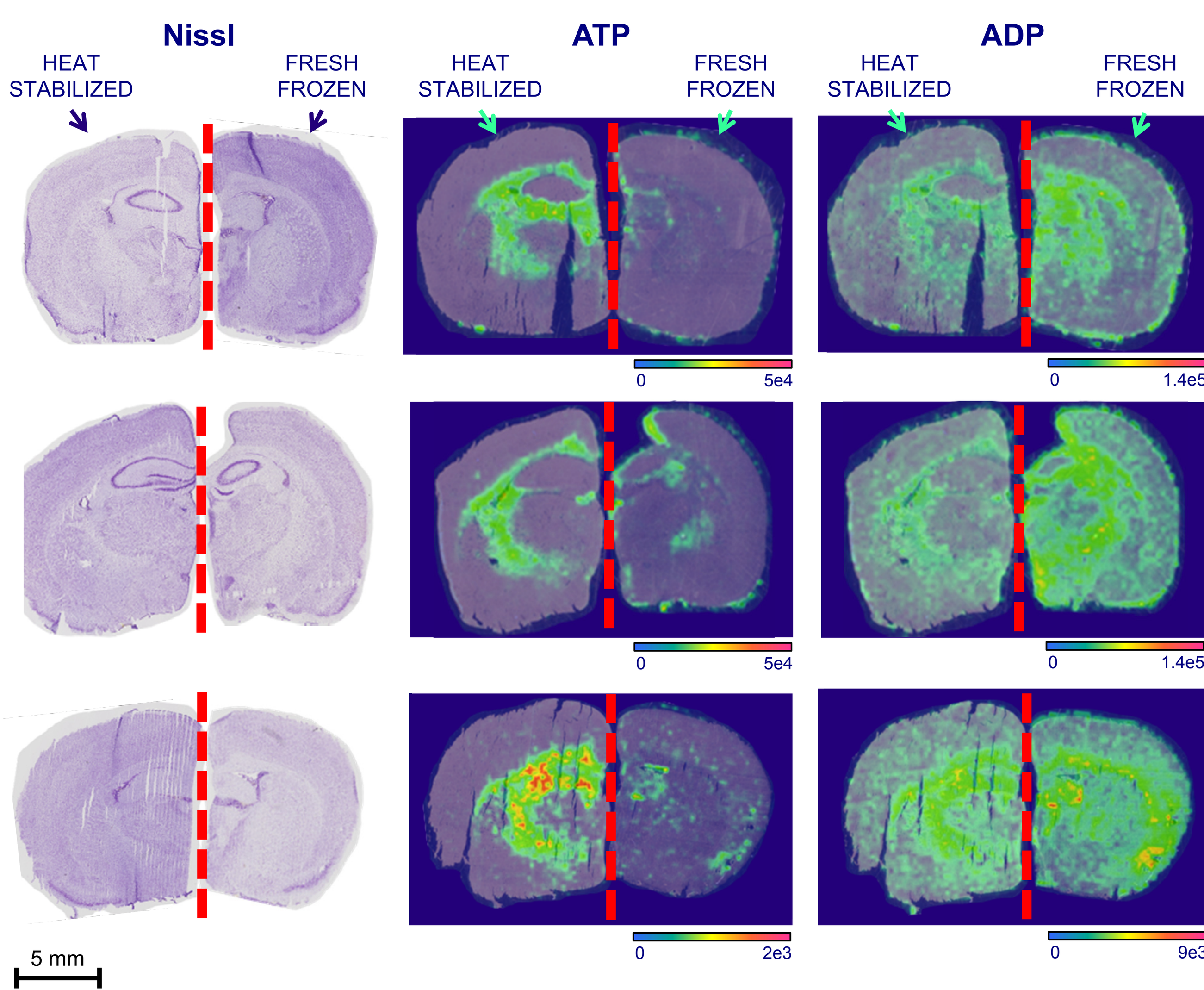
Serial sections were prepared for untargeted analyses on a 9.4 T FTICR MS (Bruker solariX). Images of fresh frozen and stabilized brains were acquired at 50 µm spatial resolution with mass accuracy typically <1 ppm. Spectra were searched manually for predicted m/z of metabolites of interest, including ATP and ADP, as well as free fatty acids, lipids, and other metabolic species.

Optimized MS^3 methods for ATP and ADP



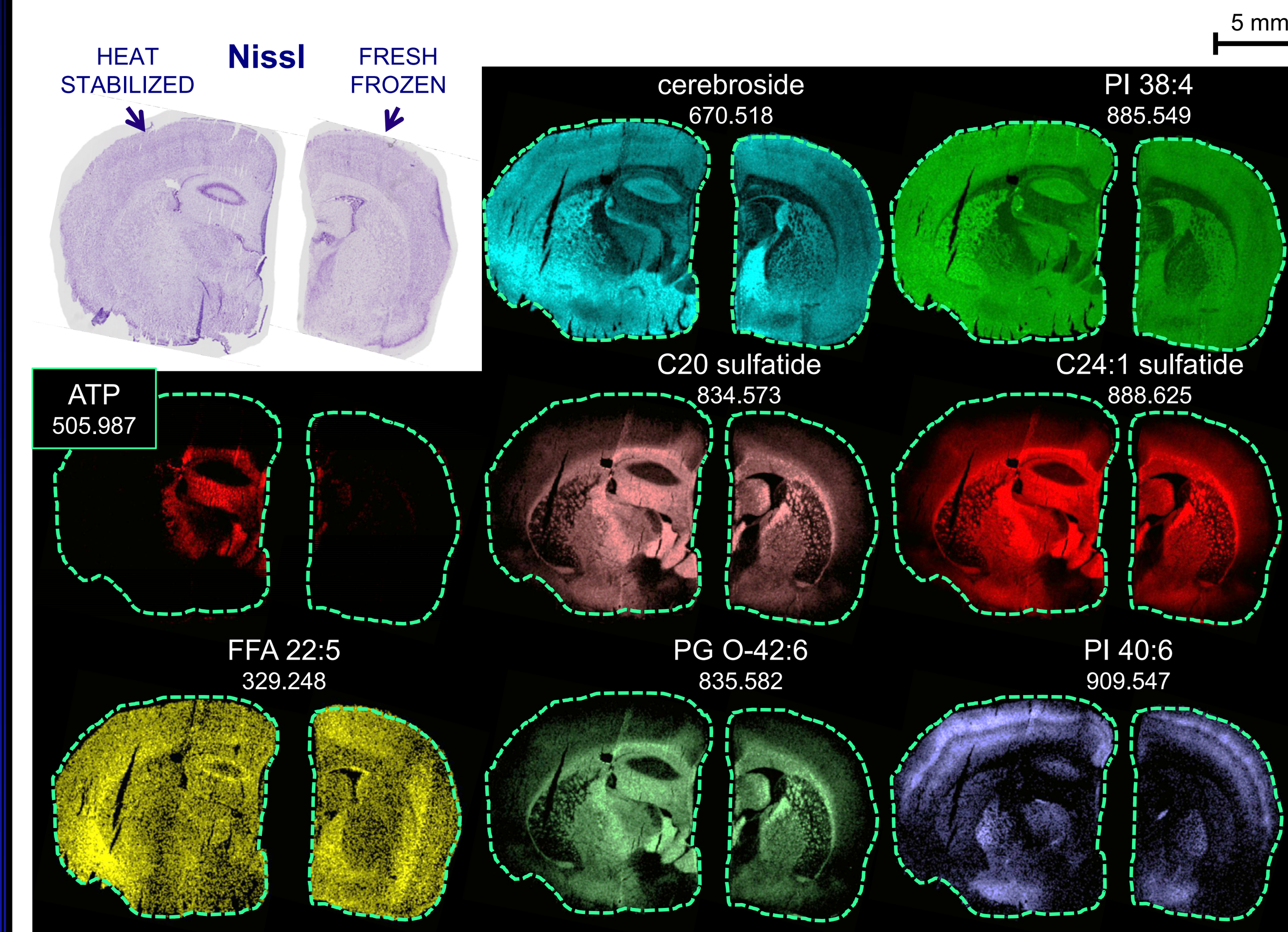
Effect of heat stabilization on ATP and ADP in rat brain

Targeted MS^3 analyses



- Inhibition of enzymatic degradation by heat stabilization results in a higher intensity of ATP and a lower intensity of ADP in stabilized tissues compared to fresh frozen

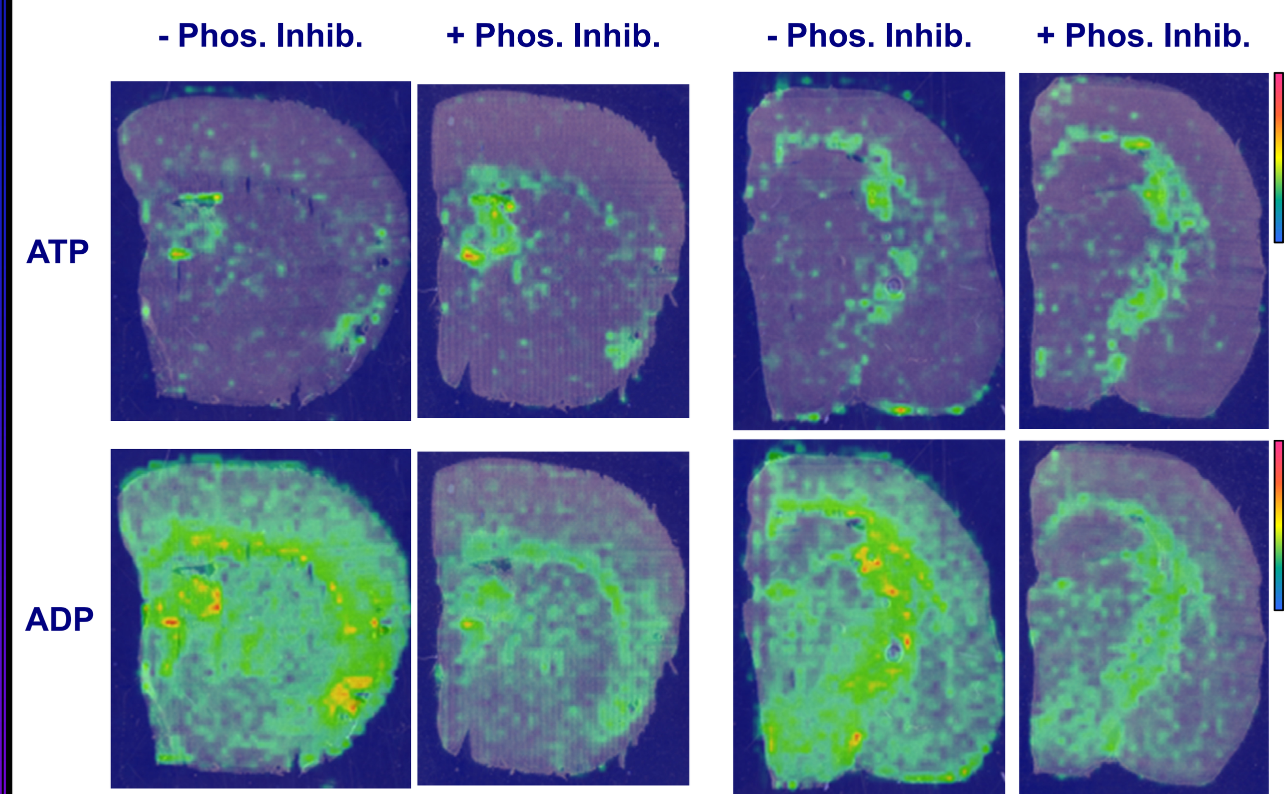
Effect of heat stabilization on other metabolites in rat brain (FTICR)



- FTICR IMS results show similar distributions for non-phosphorylated metabolites between heat stabilized and fresh frozen tissues
- Tentative identifications made based on accurate mass (typically better than 1 ppm)

Effect of phosphatase inhibitors on ATP and ADP in rat brain

Targeted MS^3 analyses



- Serial sections of two fresh frozen rat brains were thaw-mounted on MALDI target plates pre-coated with CHCA with or without phosphatase inhibitors
- An increase in ATP and a decrease in ADP is observed with the phosphatase inhibitors

Magnitude of the effects

- Ion intensities for ATP (m/z 214 + 273) and ADP (m/z 134) were summed across each tissue section
- The resulting ATP:ADP ratio was used for comparison (ImageQuest, Thermo)

Animal	Fresh Frozen	Phosphatase Inhibitor	Heat Stabilization
1	0.049	0.106	0.158
2	0.065	0.115	0.148
3	0.056	0.113	0.135
Average	0.057	0.111	0.147

- Phosphatase inhibitors resulted in ~2-fold increase in ATP:ADP compared to fresh frozen alone
- Heat stabilization resulted in ~2.5-fold increase in ATP:ADP compared to fresh frozen
- No additional effect was observed in ATP:ADP for heat stabilized tissue plus phosphatase inhibitors

CONCLUSIONS

Analysis of many small endogenous metabolites is complicated by their rapid degradation post-mortem, some of which is enzymatically driven. We have investigated two strategies to inhibit enzyme activity in dissected rat brain tissue. Rapid heat stabilization denatures proteins, thereby deactivating enzymes present in the tissue and minimizing enzymatic degradation of metabolites (and proteins) during storage and analysis. The addition of phosphatase inhibitors, similar to those added to tissue homogenates for subsequent proteomics analyses, inhibits enzyme activation upon thaw-mounting of previously frozen tissue sections. We found ~2-fold increase in the ATP:ADP ratio for serial sections of the same rat brain tissue (n=3 animals) when phosphatase inhibitors were added to matrix pre-coated onto a MALDI target plate. Similarly, we found ~2.5-fold increase in the ATP:ADP ratio for sections of heat stabilized tissue compared to fresh frozen tissue from the same animal (n = 3). This suggests both strategies are effective in suppressing phosphatase activity. Further gains in metabolite sensitivity may be obtained with improvements in sample preparation, the identity, and the quantity of enzyme inhibitors added to the target plates.

REFERENCES

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2. Srivastava, S et al, *J Neurochem*, **2012**, *123*, 688
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