

A combined approach of **imaging mass spectrometry (IMS)** and **capillary electrophoresis (CE)-MS** accomplishes region-specific metabolomics during epileptic seizure.

Yuki Sugiura and Mitsutoshi Setou
Hamamatsu University School of Medicine

Introduction

We developed a **combination technique of MALDI-IMS and CE-MS** for metabolic mapping of both abundant and trace amount of metabolite species. In our approach, (1) IMS is used to determine the tissue region where dynamic changes of abundant-metabolites were observed, (2) and then by CE-MS, wide varieties of metabolites were precisely quantified in the dissected tissue samples. Here we uncovered the region specific dynamics (i.e., how their expression fluctuates within time) of numerous metabolites during on kainate-stimulation, which is widely used as a model of limbic seizures, in the mouse hippocampus. A combined approach of IMS and CE-MS accomplished quantitative metabolomics in the small tissue regions of brain during epileptic seizure.

Methods

Kainate administration

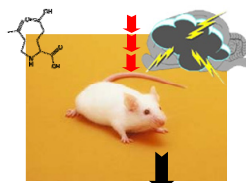


Figure 1: A combination technique of MALDI-imaging and CE-MS for metabolic mapping of bioactive metabolites.

Although MALDI-IMS provides valuable information regarding 2-dimensional distribution of metabolites, however, capability of quantitation and molecular coverage is still insufficient; the severe ion suppression effect on the tissue surface particularly disturbs the comprehensive and/or targeted analysis of interested metabolite molecules. In this regard, the highly comprehensive and quantitative analysis of charged metabolites by capillary electrophoresis mass spectrometry (CE-MS) can efficiently complement the IMS, enabling the quantification of numerous metabolites in the tissue extracts.

Frozen mouse brain

MALDI-IMS and CE-MS were used to obtain..

Two-dimensional Information

Frozen sections were cut by the cryostat at 8 um thickness.

MALDI-IMS was performed using 9-aminoacridine as the matrix (10 mg/ml, dissolved in 70% methanol) (Benabdellah, F. et al., Anal. Chem. 81, 5557, (2009)).

Imaging mass spectrometry/IMS was performed using an Ultra-Flex 2 MALDI-time of flight (TOF) mass spectrometer (Bruker Daltonics). Data were acquired in the negative-reflection mode. The interval between data points was 20 um; thus, in all, we obtained a total of approximately 6000 data points for each hippocampal region (approx. 3h measurement).

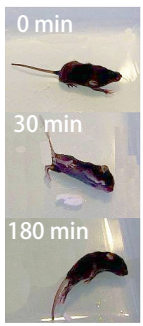
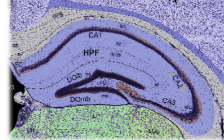


Figure 2 : Kainate induces severe epileptic seizure. Eight-week-old male C57BL/6J mice were intraperitoneally injected with kainate (25 mg/kg body weight, in saline) and then sacrificed at 30 min after dose.

Mouse hippocampus



Purine/Pyrimidine metabolism

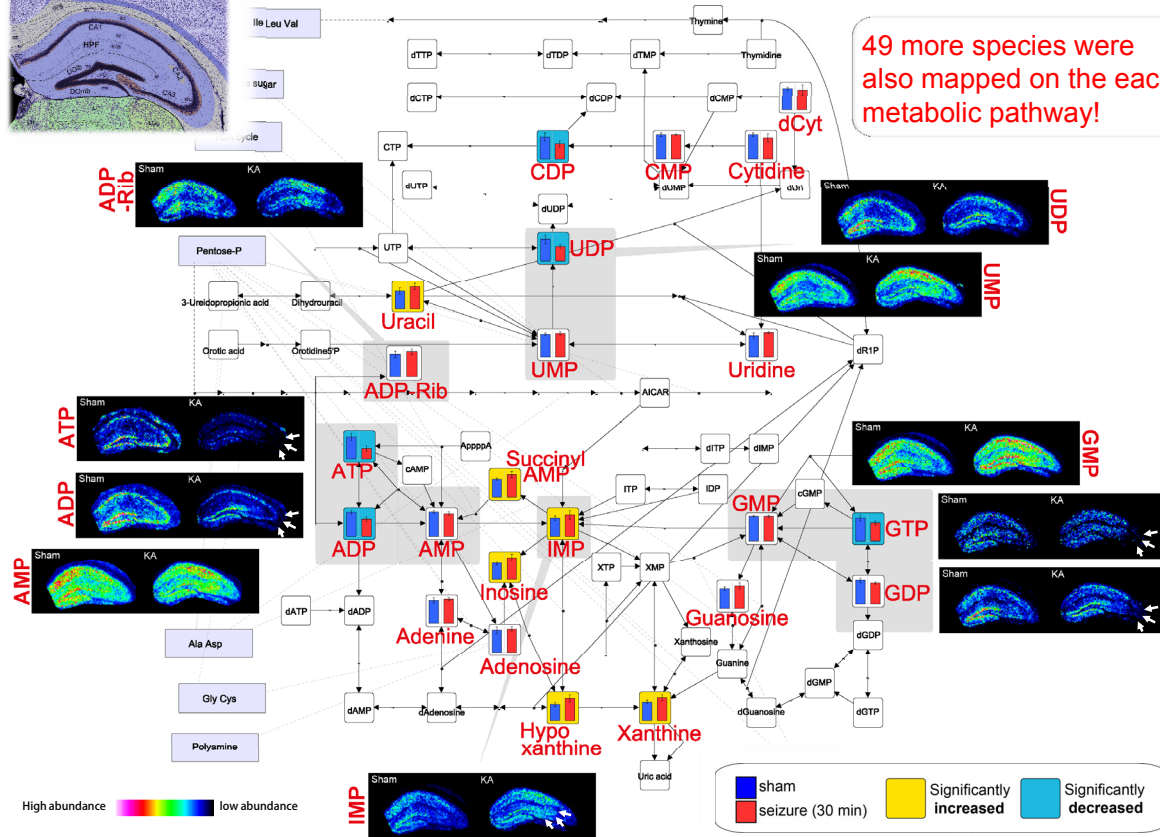


Figure 3 : Enhanced energy metabolism from purine and pyrimidine tri-/bi-phosphates during the severe seizure.

Quantitative comparison of purine/pyrimidine nucleotides by using CE-MS. In addition, corresponding MALDI-imaging results are also mapped on the pathway. By, CE-MS metabolomics, we can obtain highly quantitative data and MALDI-imaging adds the spatial data i.e., where the metabolism was enhanced. Each result is mapped on the metabolic pathway and clearly shows depletion of ATP/ADP because of their conversion into downstream metabolites.

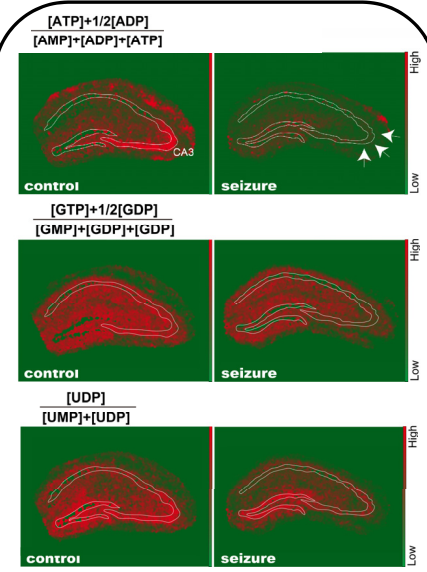


Figure 4 : Ration imaging of nucleotides

Next, we calculated the **energy-charge index** for each pixel (i.e., data point); this index was calculated as indicated equation, and represents the amount of stored cellular energy. According to the shown equation, a high index value indicates a high proportion of tri- and di-phosphated nucleotides (highly stored energy), and a low proportion of mono-phosphated ones (depletion of energy).

Pseudo-color maps of this index clearly showed enhanced metabolic turnover in the CA3 subfield (arrows). The characteristic high energy-charge index in the CA3 cell layer observed in the hippocampus of the control mice almost completely disappeared after the administration of kainate, suggesting distinct energy metabolism at the anhydride bonds of ATP/ADP in the hippocampal neurons during the seizure. Among the 3 nucleotides, adenosine nucleotides exhibited the most characteristic and prominent cellular dynamics.

Take home message

A combined approach of IMS and CE-MS accomplished 2-dimensional and quantitative metabolomics in the tissues.