PO-CON1207E

SHIMADZU
Excellence in Science

MALDI Imaging of Metabolites Reconstructed by CE-MS Based Quantitative Analysis

IMSC 2012 PMo-084

[1]Tsuyoshi Nakanishi, [2]Takako Hishiki,
[3]Shigeki Kajihara, [3]Kiyoshi Ogawa and
[2,4]Makoto Suematsu
[1]MS Business Unit, Shimadzu Corporation, Kyoto, Japan,
[2]Department of Biochemistry, School of Medicine, Keio University, Tokyo, Japan,
[3]Technology Research Laboratory, Shimadzu Corporation, Kyoto, Japan,
[4]Japan Science and Technology Agency, Exploratory Research for Advanced Technology, Suematsu Gas Biology Project, Tokyo, Japan

MALDI Imaging of Metabolites Reconstructed by CE-MS Based Quantitative Analysis

Introduction

Matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry has been widely used to visualize the spatial localization of endogenous metabolites and administered pharmaceutical drugs. In this technique, to keep high sensitivity and a good reproducibility, homogenous crystallization of matrix is required on a matrix coating onto tissue slices. As a typical matrix coating method, a spray method, a droplet method by a robotic spotter and a vapor deposition technique are known and each method is chosen according to the aim (a spatial resolution, the reproducibility, an extraction efficiency of metabolites, a cost, etc.). In this study, we performed matrix deposition by usage of a chemical printer as a robotic spotter, to keep the reproducibility of ion images from endogenous metabolites between multiple samples.

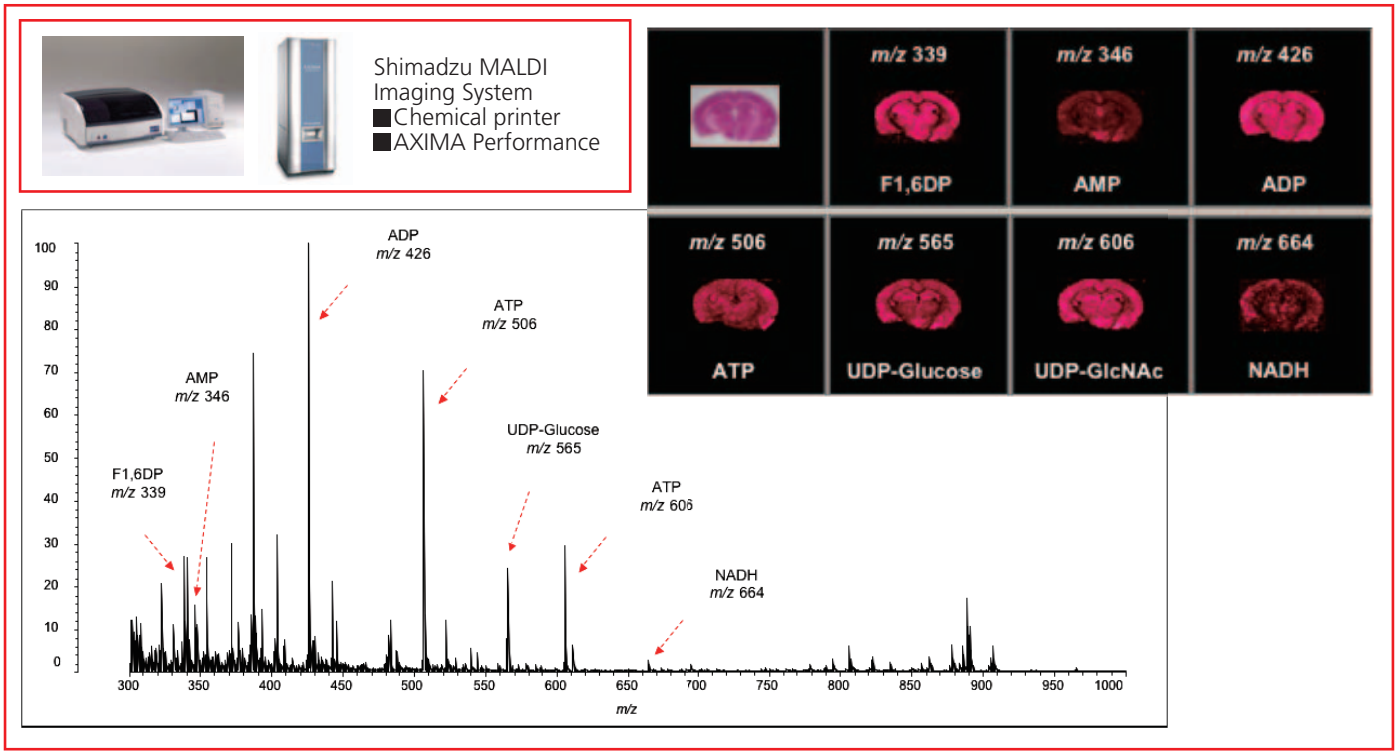


Fig. 1 Typical mass spectrum and mass image of metabolites on a mouse brain tissue

Subsequently, to reflect the concentration of metabolites in tissues to the molecular images, the quantitative data by CE-MS measurement was coupled to ion images of these metabolites obtained by imaging mass spectrometry (IMS) from a serial section. Here we quantified ATP and its degradation metabolites, ADP and AMP in a murine brain tissue by CE-MS and reconstructed ion images to semiquantitatively evaluate the changes of energy metabolism among the multiple samples. By using this semiquantitative approach, we displayed the distribution of AMP, ADP and ATP in a brain tissue from both wild type and transgenic mice deficient with a target enzyme under the normoxia/hypoxia.
Furthermore, energy charge (EC) was also calculated and visualized by the reconstructed molecular images of energy metabolites.

MALDI Imaging of Metabolites Reconstructed by CE-MS Based Quantitative Analysis

Methods

Imaging mass spectrometry (IMS)

Murine brain tissue (10 µm thick) was prepared using *in situ* freezing method[1]. A 9-aminoacridine was selected as a MALDI-matrix and microdispensed onto frozen brain slices as a spatial resolution at 200 µm by a chemical inkjet printer (CHIP-1000, Shimadzu Corp.). Imaging mass spectrometry was performed in negative mode by MALDI-TOF/TOF MS instrument (AXIMA Performance) on the basis of positional information of each matrix deposit. Ion images were reconstructed by BioMap software after TIC normalization.

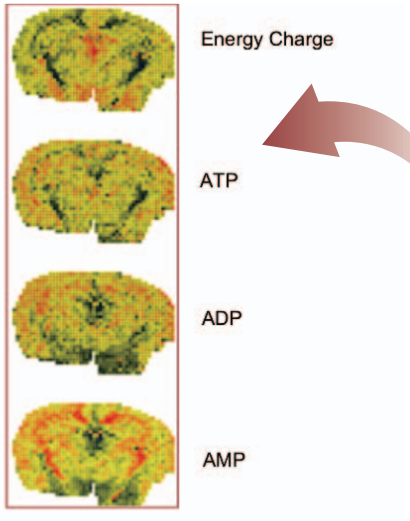
Quantification of metabolites by CE-MS

Frozen brain sections from mice were plunged into ice-cold methanol containing internal standards and homogenized with a polytron homogenizer. After chloroform/methanol extraction, the upper aqueous layer was centrifugally filtered through a 5-kDa cutoff filter.
Quantification of metabolites was performed as described previously[1].

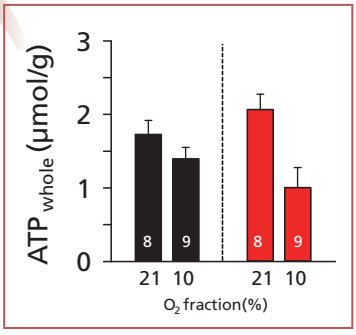
Coupling of quantitative data to mass images of metabolites

To construct metabolite mapping between different slices, the quantitative data by CE-MS was linked to the IMS data of each metabolite. Here we estimated the apparent concentration (C_i) of each metabolite at the *i*th spot (corresponding to the ith pixel on a mass image) of tissue as follows: $C_i = C' \times Int_i/Int_{ave}$, where C', means the metabolite concentration of tissue determined by CE-MS quantification, Int_i is the intensity of a target metabolite on a mass spectrum at the *i*th spot, and Int_{ave} is the median of intensities of the metabolite from all of the spots. Thus metabolite maps (AMP, ADP and ATP) were reconstructed to evaluate the fluctuation of energy metabolism under the normoxia/hypoxia using transgenic mice deficient with a target enzyme. Furthermore, energy charge (EC) was also calculated as:

$$EC=([ATP]+1/2[ADP])/([ATP]+[ADP]+[AMP])$$



Metabolite concentration determined by CE-MS was coupled to MS images



$$\text{Energy Charge} = \frac{([ATP]+1/2[ADP])}{([ATP]+[ADP]+[AMP])}$$

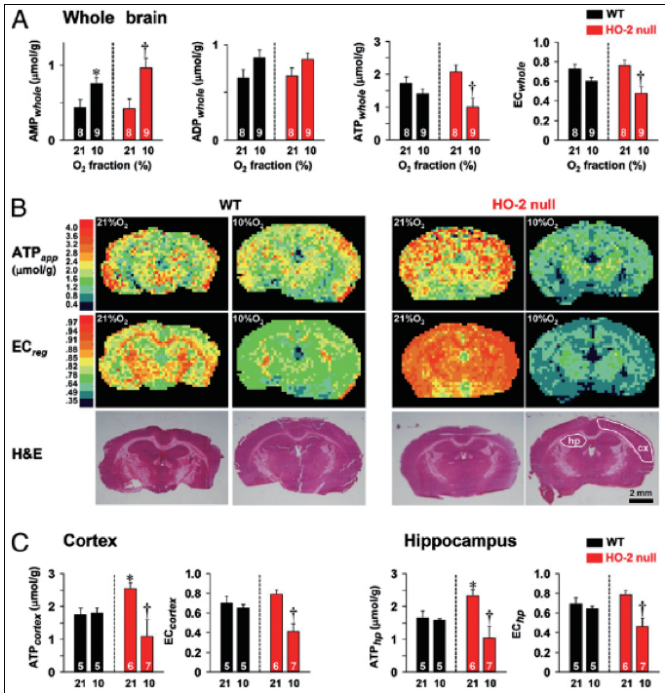
Fig. 2 shows reconstructed mass images of AMP, ADP, ATP and EC map which was calculated from the three metabolites (AMP, ADP and ATP). Metabolite levels were quantified by CE-MS and the information about the concentration in tissues was used to reconstruct the metabolite maps. Here the apparent concentration (C_i) of each metabolite at the *i*th spot was calculated as $C_i = C' \times Int_i/Int_{ave}$ (see method section).

Fig. 2 Metabolite maps reconstructed from the quantitative data by CE-MS.

Results

Molecular images of typical metabolites were visualized on mouse brain tissue (10 μm thick) using 9-aminoacridine as a MALDI-matrix. Then chemical inkjet printer was used to keep a homogenous matrix deposition onto tissue slices and resulted to reproducible ion images between multiple samples.

By using the IMS results coupled to the CE-MS based quantitative data, metabolite maps were reconstructed among brain tissue slices from the different individuals. The metabolite maps which were reconstructed on the basis of the quantitative results by CE-MS, reflect the endogenous concentration of each metabolite. In fact, AMP, ADP and ATP which related to energy metabolism were visualized from tissue slices under the normoxia/hypoxia and these ion images displayed an unique alteration on both the distribution and the content.



(A) Alterations in AMP (AMPwhole), ADP (ADPwhole), ATP (ATPwhole), and energy charge (ECwhole) in the whole brain. The concentrations of adenylates were determined by CE-MS. *P < 0.05 compared with WT normoxia; †P <0.05 compared with HO-2-null normoxia. HO-2, heme oxygenase 2

(B) Representative IMS showing spatial distribution of apparent ATP concentration (ATPapp) and energy charge (ECreg). Note the basal increase in ATP in HO-2-null mice. (Bottom) H&E staining after IMS. cx, cortex; hp, hippocampus.

Fig. 3 Impaired ability of HO-2-null mice to maintain ATP levels on exposure to 10% O_2 for 1 min

Furthermore, when mice were exposed to 10% O_2 for 1min as a hypoxia, rapid decrease of ATP level was observed in a brain of HO-2-null mice. These results suggest this developed approach is useful to semiquantitatively evaluate the distribution of metabolites on the IMS experiment.

References

(1) Hattori K, et al. (2010) Paradoxical ATP elevation in ischemic penumbra revealed by quantitative imaging mass spectrometry. *Antioxid Redox Signal* 13:1157-1167.

(2) Morikawa T, et al. (2012) Hypoxic regulation of the cerebral microcirculation is mediated by a carbon monoxide-sensitive hydrogen sulfide pathway. *Proc. Natl. Acad. Sci. U.S.A.* 109:1293-1298.23

First Edition: September, 2012

Shimadzu Corporation
www.shimadzu.com/an/