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Metabolite maps reconstructed using a quantitative data by capillary electrophoresis-mass spectrometry (CE-MS)

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Overview

Imaging mass spectrometry is a powerful tool to display the unique distribution of endogenous metabolites in tissue slices and this technique is currently used to the wide variety of animal and plant systems. Here to reflect the content of metabolites in tissues to the MS images, the quantitative data by CE-MS was to tried to couple to the results of MALDI imaging experiment. Metabolite maps were reconstructed by the information of each metabolite quantified from the CE-MS measurement.

Introduction

Imaging mass spectrometry (IMS) provides information of metabolite localization or distribution of a dosed drug in tissue sections. In general IMS technique requires a homogeneous matrix coating for a good reproducibility. This procedure also influences variable quantitative results on IMS technique. To improve these drawbacks, we achieved a reproducible matrix deposition by usage of a robotic spotter and coupled quantitative results of metabolites by CE-MS with ion images obtained by IMS to reconstruct metabolite maps from different slices. Here we quantified ATP and its degradation metabolites, ADP and AMP, using CE-MS in brains from both wild type or transgenic mice deficient with a target enzyme. Furthermore, we examined a regional variation of metabolites in energy metabolism under normoxia/hypoxia by this method.

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 by CE-MSD 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:

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

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Results

A mouse coronal brain tissue was prepared according to *in situ* freezing method to maintain a level of ATP and ADP. Tissue slices were placed onto a conductive slide glass and then dried up. A 9-aminoacridine matrix was microdispensed onto tissue slices at a spatial resolution at 200 mm by a robotic spotter. A mass spectrum was acquired from each matrix spot onto tissue slices by MALDI-TOF/TOF instrument (AXIMA Performance, Shimadzu Corp.). Fig. 1 shows a typical mass spectrum from a murine brain tissue when using 9-aminoacridine as a MALDI-matrix. Metabolites with phosphate group and phospholipids were observed on this mass spectrum.

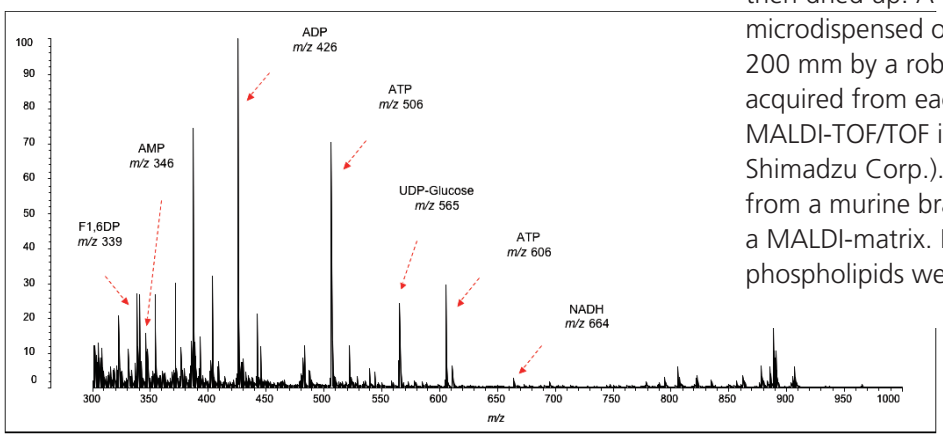
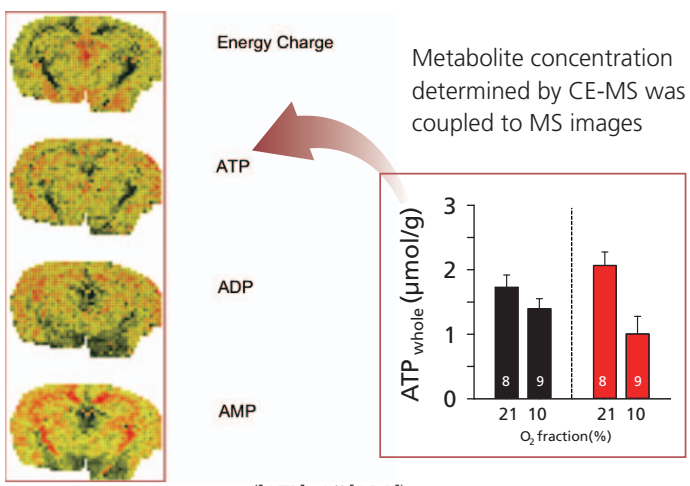


Fig. 1 Typical mass spectrum from wild-type murine brain tissue using 9-aminoacridine as a MALDI-matrix

Typical mass image of metabolites were displayed from obtained mass spectra by BioMap software (Fig. 2). To evaluate variation of metabolites between multiple samples from different individuals, we performed TIC normalization with in-house designed software. Furthermore, to display metabolite maps corresponding to a content of metabolites in tissue, we tried to couple the quantitative data by CE-MS to mass images of each metabolite (see Fig. 3).

Fig. 2 Typical mass image of metabolites using 9-aminoacridine as a MALDI-matrix



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

The concentration of metabolites quantified by CE-MS was coupled to the IMS data and apparent concentration (on tissue) of each metabolites was estimated on MS images. Fig. 3 shows mass images of AMP, ADP, ATP and EC map which was calculated from the three metabolites (AMP, ADP and ATP). First metabolite levels were quantified by CE-MS and the information about the metabolite concentration in tissue was used to reconstruct the metabolite maps. Here the apparent concentration (Ci) of each metabolite at the ith spot was calculated as $Ci=C' \times Inti/Intave$ (see method). We could reproducibly compare both the distribution and abundance of metabolites between multiple tissues from the different individuals.

Fig. 3 Metabolite maps reconstructed from the quantitative data by CE-MS

Metabolite maps reconstructed using a quantitative data by capillary electrophoresis-mass spectrometry (CE-MS)

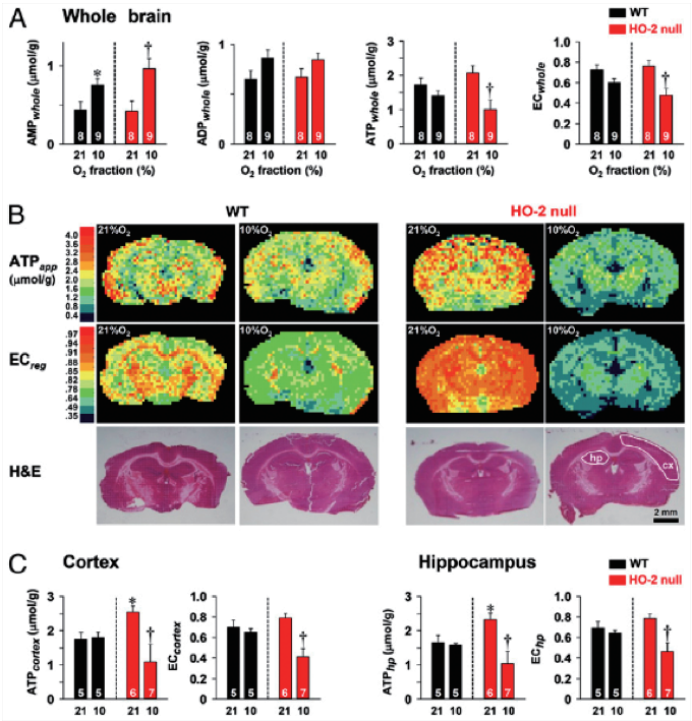


Fig. 4 Impaired ability of HO-2-null mice to maintain ATP levels on exposure to 10% O2 for 1 min

(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.

(C) Quantitative analysis of regional ATP concentration and energy charge in WT and HO-2-null mice. *P < 0.05 compared with WT normoxia; †P < 0.05 compared with HO-2-null normoxia.

From results of quantitative analysis by CE-MS, remarkable decrease of ATP level in HO-2-null mice under the hypoxia was shown in Fig. 4 Furthermore, a value of EC under the hypoxia remained unchanged in WT mice but dropped to <0.5 in the HO-2-null mice.
IMS data coupled by CE-MS quantitative results also displayed the characteristic decrease of EC value on the cortex region.

Conclusions

MS images of typical metabolites were displayed on mouse brain tissue (10 µm thick) using 9-aminoacridine as a MALDI-matrix. Then chemical inkjet printer was used for homogenous matrix deposition onto tissue slices and resulted to reproducible MS images between multiple samples.
By using the IMS data coupled to the quantitative data of CE-MS measurement, metabolite maps were reconstructed using 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 detected from tissue slices under the normoxia/hypoxia and these MS images displayed an unique alteration on the distribution and the content in WT and a transgenic mouse. These results suggests this developed approach is useful to semiquantitatively evaluate the distribution of metabolites on the IMS experiment.

References

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