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Application News

No. B87

Probe Electrospray Ionization Mass Spectrometer

Establishment of a Method for Direct Analysis of the Mouse Brain Metabolome Using the DPiMS™-8060

Metabolome analysis is a method for comprehensively analyzing endogenous metabolites such as amino acids, organic acids, fatty acids, and sugars, and in recent years has come to be widely used in the field of life science. Probe electrospray ionization (PESI) is a direct ionization method in which an ultrafine and minimally invasive probe is used for sampling. Acquired samples are ionized by applying a high voltage to the probe tip and therefore components can be analyzed without a chromatograph. By using the DPiMS-8060 probe electrospray ionization tandem mass spectrometer (Fig. 1), which combines PESI with tandem mass spectrometry, direct analysis of the metabolome of a biospecimen is possible.

Generally, pretreatment is indispensable for the metabolome analysis of a brain sample since there are many lipids in the brain which cause interference in analysis. However, by using the DPiMS-8060, we succeeded in a direct and rapid analysis of metabolites in a brain sample without any pretreatment.

This article introduces a method for analyzing brain metabolites using a PESI tandem mass spectrometer.

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Fig. 1 **DPiMS™-8060**

Analysis of the Mouse Brain Metabolome

This research attempted direct analysis of brain metabolites without any complex pretreatment by using the newly developed DPiMS-8060 mass spectrometer which combines probe electrospray ionization (PESI), a novel ionization method that uses an ultrafine probe, with tandem mass spectrometry.

Standard metabolite samples including amino acids, organic acids, and sugars (25 metabolites) were prepared by diluting them with 50 % ethanol solution and dripping 10 µL of each sample into sample plates for liquid samples. We then selected the MRM transitions for each compound and optimized the mass spectrometer conditions such as collision energy (CE). The resulting values are listed in Table 1.

Table 1 **MRM Transitions of 25 Metabolites**

Name	Polarity	Transition (*m/z*)	Collision Energy (V)
3-hydroxybutyrate	(–)	103.1>59.0	35
Citric acid/isocitric acid	(–)	191.3>111.2	20
D-glucose	(–)	179.0>89.1	20
Glucose-6-phosphate	(–)	259.1>96.9	20
Glutaric acid	(–)	131.0>87.3	20
Glycine	(–)	74.2>74.2	20
L-asparatic acid	(–)	131.9>88.1	20
L-glutamic acid	(–)	146.0>102.1	20
L-lactic acid	(–)	89.0>43.2	20
L-malic acid	(–)	133.0>114.9	20
L-serine	(–)	103.9>74.2	20
N-acetyl-L-asparate	(–)	174.0>88.2	20
Pyruvic acid	(–)	87.1>43.1	20
Succinic acid	(–)	117.1>73.0	20
Uracil	(–)	111.0>41.8	20
α-ketoglutaric acid	(–)	144.6>100.8	20
L-histidine	(–)	154.0>93.1	20
L-methionine	(–)	147.9>46.9	20
L-phenylalanine	(–)	164.2>147.0	20
L-threonine	(–)	118.1>74.1	20
L-tryptophan	(–)	203.3>116.0	20
2-aminobutyric acid	(+)	104.1>58.1	35
GABA	(+)	104.2>45.0	20
L-glutamine	(+)	147.1>84.2	20
L-leucine/L-isoleucine	(+)	132.1>86.2	20

Next, we rapidly analyzed brain metabolites (0.2 min per analysis) without any pretreatment. The metabolites in the frontal cortex of a model group of mice administered a drug to disrupt energy metabolism in the brain (AM-2201) and a control group of mice were analyzed by DPiMS/MS. Multivariate analysis (PLS-DA) results are shown in Fig. 2. Since the metabolome of the model group and the control group is clearly separated as shown in Fig. 2 (a), we can see that the metabolome profile differs between the groups. Using the loading plot shown in Fig. 2 (b), it is possible to identify the metabolites that differ greatly between the two groups. In this case, we can see that metabolites such as glutamic acid and succinic acid differ greatly. These results match well with the metabolome analysis results using a GC/MS/MS system introduced previously, indicating that the DPiMS-8060 is capable of obtaining metabolome analysis results of the same level as with existing methods even without any pretreatment.

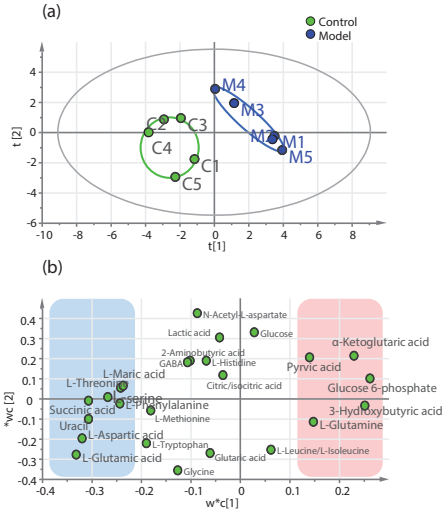

Fig. 2 **Results of Multivariate Analysis of Frontal Cortex Metabolites of the Control Group and the Model Group**

Since the probe used for sampling and ionization is ultrafine with a tip diameter of about 700 nm, the DPiMS-8060 may be applicable to distribution analysis of extremely minute areas. We next therefore analyzed the metabolites in the frontal cortex and the hippocampus using the DPiMS-8060. The results are shown in Fig. 3. As indicated by the results, direct analysis of the difference in the local distribution of frontal cortex and hippocampus metabolites was successful without pretreatment. Upon evaluating matrix effects regarding the frontal cortex and hippocampus using glutamic acid labeled with a stable isotope, no significant difference was observed between the two (Fig. 4).

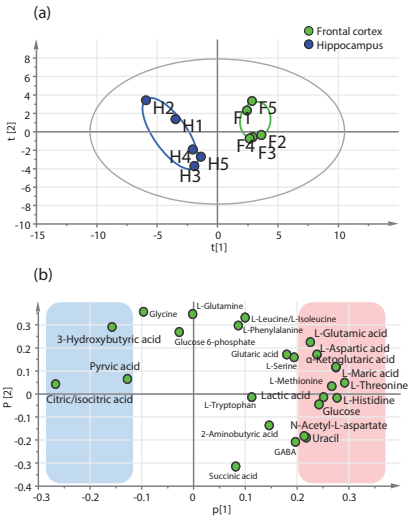

Fig. 3 **Results of Multivariate Analysis of Metabolites in the Frontal Cortex and Hippocampus**

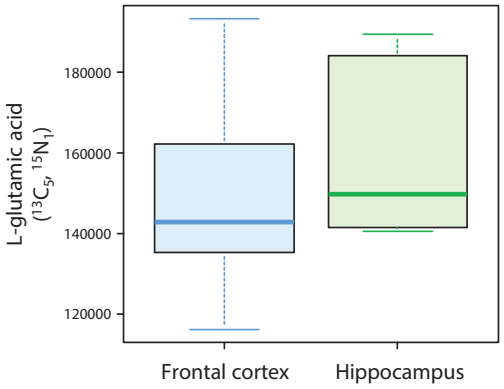

Fig. 4 **A Comparison of Matrix Effects Regarding the Frontal Cortex and Hippocampus**

Conclusion

We confirmed that brain metabolites can be detected very easily and rapidly without any complex pretreatment by using the new DPiMS-8060 mass spectrometer, even for brain samples with high lipid content.

In addition, by taking advantage of the fact that an ultrafine probe is used, local analysis of brain metabolites is also possible.

Acknowledgments

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References

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