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Application News

No. C192

Liquid Chromatography Mass Spectrometry

Simultaneous Analysis of Chiral Amino Acids Produced by Intestinal Microbiota using LC/MS/MS

Some of the metabolites produced by intestinal microbiota are constantly absorbed from the intestinal lumen and transported throughout the entire body. In recent years, it has become clear that the intestinal microbiota contributes to the preservation and promotion of the hosts' health and attention has been focused on the relationship of the intestinal microbiota and metabolites with health and diseases. For this reason, it is extremely important to analyze the metabolites produced by the intestinal microbiota.

D-amino acids are among the low molecular weight metabolites, and their physiological functions differ from those of L-amino acids. Amino acids have optical isomers (the D-isomer and L-isomer) and it has long been considered that only L-isomers are involved in the biological world. In recent years, however, it has been found that there are D-amino acids in the bodies of mammals, and their functions are attracting attention. To give an example, it is suggested that there is a relationship of D-serine with schizophrenia and amyotrophic lateral sclerosis.

In this study, we comprehensively analyzed chiral amino acids in mouse colonic contents and blood plasma, using LC-MS/MS, and investigated D-amino acids produced by the intestinal microbiota.

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Sample Pretreatment

The mouse colonic contents and blood plasma used in the analysis were taken from germ-free (GF) mice and mice with a normal bacterial flora established (Ex-GF). The pretreatment protocol is shown in Fig. 1.

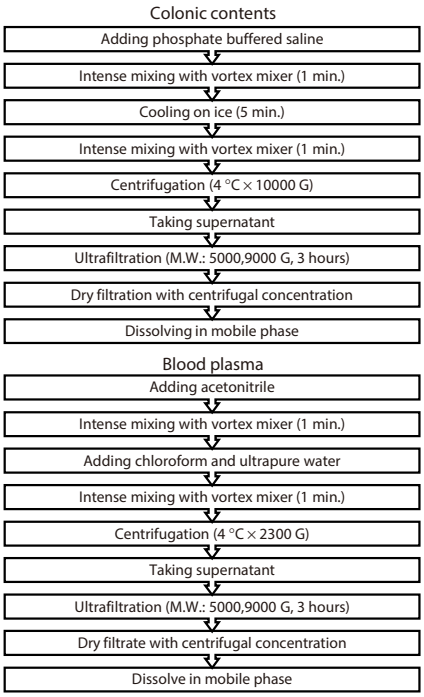

Fig. 1 Pretreatment Protocols for Mouse Colonic Contents and Blood Plasma

Analysis Conditions

Table 1 shows the analysis conditions. In this analysis, we used the LC/MS/MS Method Package for D/L Amino Acids and LCMS™-8060, which are shown in Fig. 2. The conventional chiral amino acid analysis required derivatization of the amino acids and long time for separation. Using this method package makes it possible to accomplish the analysis in a short time with no need to derivatize each chiral amino acid, so the analysis can proceed efficiently. For details, refer to Application News Nos. C149 and C156.

Table 1 Analysis Conditions

UHPLC (Nexera™ X2 system)	
Column	: CROWNPAK® CR-I (+) / CR-I (–) (3 mm × 150 mm, 5 µm, DAICEL Corp.)
Mobile phase	: acetonitrile/ethanol/water/trifluoroacetic acid = 80/15/5/0.5
Flow rate	: 0.6 mL/min
Column temp.	: 25 °C
Injection volume	: 1 µL

MS (LCMS™-8060)	
Ionization	: ESI positive
Nebulizing gas flow	: 3.0 L/min
Drying gas flow	: 15.0 L/min
Heating gas flow	: 5.0 L/min
Interface temp.	: 250 °C
DL temp.	: 250 °C
Block heater temp.	: 300 °C

Fig. 2 LC/MS/MS Method Package for D/L Amino Acids and the Nexera™ X2 + LCMS™-8060 System

Results

Some representative results from among the MRM chromatograms obtained by analysis are shown in Fig. 3. It can be seen from this figure that D- and L-amino acids are separated adequately under these analysis conditions, and that D-serine and D-alanine are detected in the colonic contents of the Ex-GF mice, which have bacteria, but not detected in GF mice, which have no bacteria.

Next, the results of analysis of the colonic contents and blood plasma of GF mice and Ex-GF mice are shown in Fig. 4. It presents the quantitative values for each amino acid in graph form using the Shimadzu Multi-omics Analysis Package to allow comparison of the analysis results for D- and L-amino acids contained in each sample. From the results of the analysis of the colonic contents, 7 types of D-amino acids were detected in GF mice, and 14 types in Ex-GF mice. Among these, the concentration of 12 types of D-amino acids (alanine, arginine, aspartic acid, glutamine, glutamic acid, allo-isoleucine, leucine, lysine, methionine, phenylalanine, serine, tryptophan) were significantly high in Ex-GF mice, so it was determined that these D-amino acids were produced by the intestinal microbiota. In blood plasma, 3 types of D-amino acids were detected in GF mice and 6 types in Ex-GF mice. Furthermore, the D-alanine concentrations in the colonic contents and blood plasma in the ex-GF mice were significantly higher than those in the GF mice, which suggested that the D-alanine produced by the intestinal microbiota had migrated into the blood.

Conclusion

This study indicates that it is possible to quantify D- and L-amino acids in biological samples quickly and with high sensitivity by using LC/MS/MS, which allows comprehensive analysis without derivatization, in combination with the LC/MS/MS Method Package for D/L Amino Acids. We expect that this analytical method will be rolled out to further research into physiological functions of D-amino acids and disease prevention.

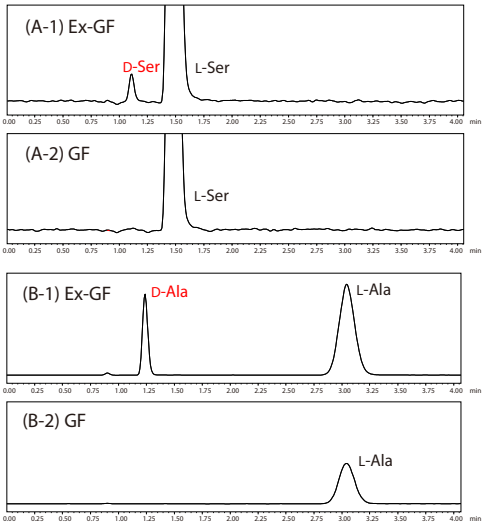

Fig. 3 MRM Chromatograms Obtained from the Analysis of Colonic Contents
(A-1): D-/L-Serine among Ex-GF Colonic Contents
(A-2): D-/L-Serine among GF Colonic Contents
(B-1): D-/L-Alanine among Ex-GF Colonic Contents
(B-2): D-/L-Alanine among GF Colonic Contents

<References>
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Fig. 4 D- and L-amino Acid Concentrations in Colonic Contents and Blood Plasma (Red: GF, Yellow: Ex-GF)

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