SHIMADZU
Excellence in Science

Application News

No. C188

Liquid Chromatography Mass Spectrometry

Analysis of Galactolipids of Eukaryotic Algae by LC/MS/MS

Galactolipids are a type of glycolipid, which accounts for nearly 80 % (molar ratio) of the chloroplast membrane lipids of higher plants and is found abundantly in nature. Instead of using phospholipids, plants and other photosynthetic autotrophs use galactolipids, which do not contain phosphorus, as the major component of the thylakoid membrane of their chloroplasts.

The following significant findings on the biological activities of galactolipids have been obtained by researches conducted in the past few decades:

1. Direct involvement in thylakoid biosynthesis
2. Indispensable for photosynthetic electron transport reaction
3. Partially substitutes for phospholipids during phosphorus starvation
4. The role in flower development
5. Contribution to the anti-inflammatory properties and antitumor activities of medicinal plants

A galactolipid is composed of at least one galactose head group attached to the sn-3 position of the glycerol backbone via an ether bond and two fatty acid chains attached to the sn-1 position and sn-2 position via an ester bond (Fig. 1). Galactolipids are amphiphilic because galactose has a hydroxyl group.

Galactolipids are classified by the types of sugars into groups, including monogalactosyl diacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), and sulfoquinovosyldiacylglycerol (SQDG). The structures of MGDG and DGDG, the major galactolipids, are shown in Fig. 1. The ratio of MGDG and DGDG as well as their fatty acid composition and distribution are important indicators for research on chloroplast formation.

Euglena gracilis are one type of euglenids which are a group of eukaryotic unicellular algae; they have chloroplasts and can proliferate photoautotrophically and heterotrophically. As wax esters formed in Euglena gracilis under low oxygen conditions are considered as excellent raw materials for biofuel production, Euglena gracilis have been gaining more attention in recent years.

This article introduces an example analysis of galactolipids in Euglena gracilis powder using a high performance liquid chromatograph and triple quadrupole mass spectrometer system (Shimadzu LCMS™-8060), as part of analyzing lipids of Euglena gracilis.

H. Lin

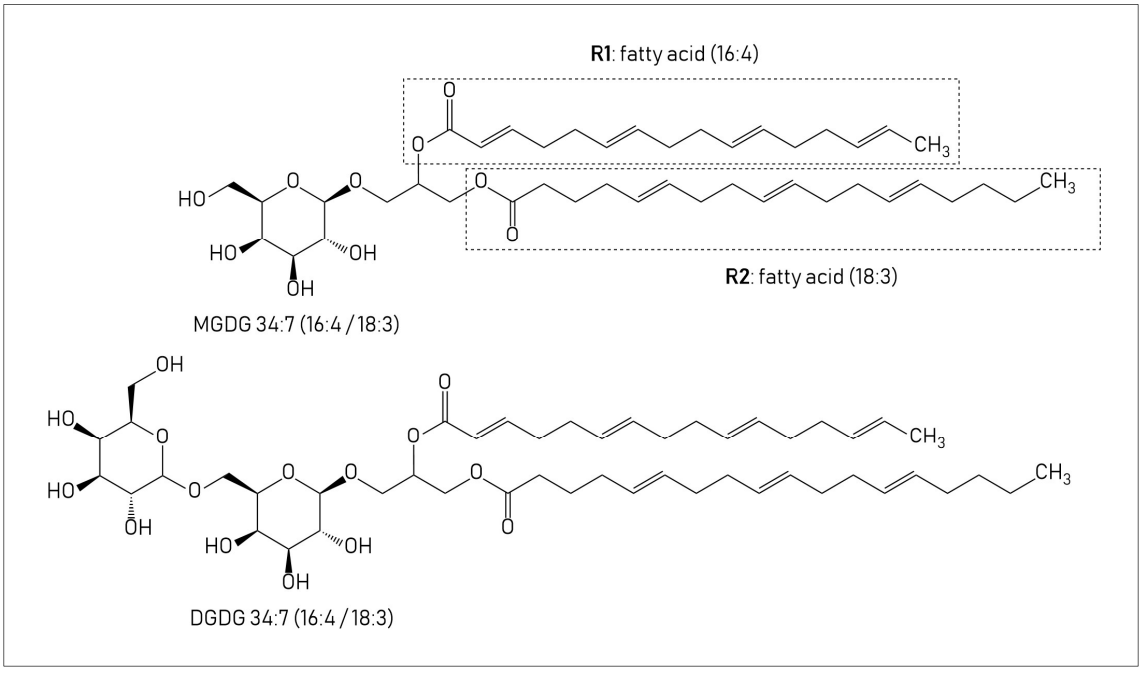

Fig. 1 Structures of MGDG 34:7 (16:4 / 18:3) and DGDG 34:7 (16:4 / 18:3)
(Double-bond Positions and Geometric Isomers of these Fatty Acids Not Identified)

Sample Pretreatment

The dried powder of large-scale cultivated Euglena gracilis was provided by Euglena Co., Ltd.

A volume of 600 µL of a 1:1 solution of methanol and chloroform was added to 3 to 5 mg of the sample powder, and the mixture was agitated for 5 minutes into a suspended form. Subsequently, 300 µL of ultra-pure water was added to the mixture, and the mixture was agitated for 10 minutes. After leaving the mixture for 10 minutes at room temperature, it was centrifuged at 20,000 g for 10 minutes at 4 °C and the organic phase (bottom phase) was collected. The collected sample was diluted by a factor of 100, and 5 µL of the diluted sample was subjected to LC-MS/MS analysis.

Table 1 Analysis Conditions

[LC] Nexera™ X2 System		
Column	:	Phenomenex Kinetex C8 (2.1 mm I.D. × 150 mm, 2.6 µm)
Solvent A	:	20 mM ammonium formate
Solvent B	:	2-propanol / acetonitrile (1:1)
Flow rate	:	0.3 mL/min
Column temp.	:	45 °C
Injection vol.	:	5 µL
Gradient (B conc.)	:	20 % (1 min) – 92.5 % (25 min), curved gradient (–3)
[MS] LCMS-8060		
Ionization	:	ESI negative
DL temp.	:	250 °C
Heat Block temp.	:	400 °C
Interface temp.	:	150 °C
Nebulizer gas	:	2.0 L/min.
Drying gas	:	10.0 L/min.
Heating gas	:	10.0 L/min.

MRM Transition

The results of the analysis of MGDG and DGDG standards (catalog No.: 840523/840524, Avanti Polar Lipids) revealed that MGDG and DGDG are detected as formate adducts mainly in the negative mode. As the result of product ion scanning with the formate-adduct ion as the precursor ion, the deprotonated molecules were the product ions having a high intensity that were detected stably. In consideration that the fatty acids of galactolipids have the total number of carbon atoms of 32 to 44 and the total number of double bonds of 0 to 12, an MRM transition library was constructed from 91 types of MGDG and 91 types of DGDG (total: 182 types) with the formate-adduct ion as the precursor ion and the deprotonated molecules as the product ions.

Analysis Results

The signal intensities and the results of the separation of galactolipids detected in the lipid extract from Euglena gracilis are shown in Fig. 2. The mass chromatograms of MGDG and DGDG with 34-carbon fatty acids and a total of 2 to 7 double bonds (the most abundant components) are shown in different colors.

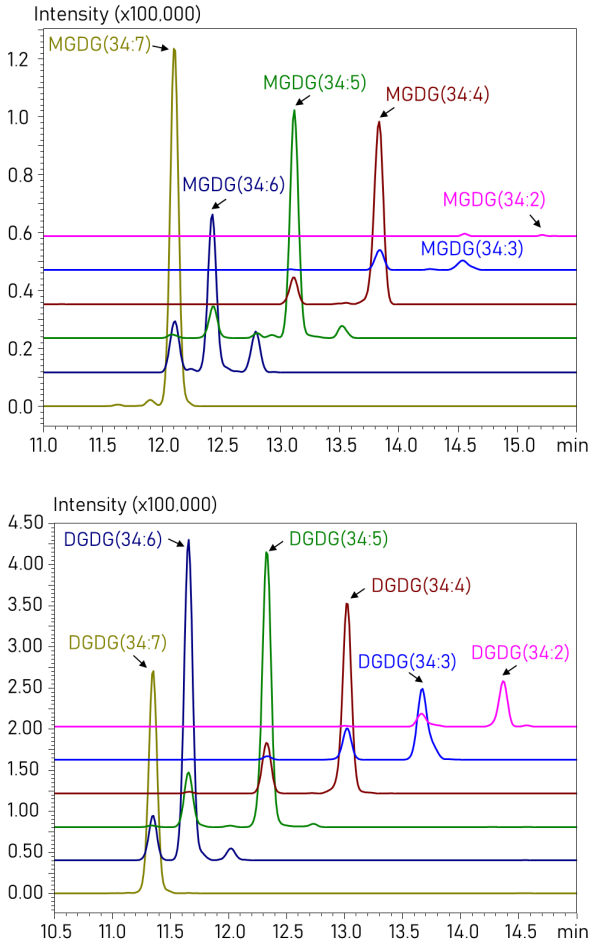

Fig. 2 Separation and Detection of MGDG and DGDG in the Lipid Extract from Euglena Gracilis Using LCMS-8060

<Acknowledgments>
We would like to express our sincere appreciation to Dr. Takuya Matsumoto of the Central Research Center of Euglena Co., Ltd. for providing the Euglena samples and for the valuable discussions.

First Edition: Mar. 2019

Shimadzu Corporation
www.shimadzu.com/an/

Related Products Some products may be updated to newer models.

LCMS-8060

High Performance Liquid Chromatograph Triple Quadr···

Related Solutions

- Price Inquiry
- Product Inquiry
- Technical Service / Support Inquiry
- Other Inquiry