Chapter 6

LIPIDOMICS: ESI-MS/MS-BASED PROFILING TO DETERMINE THE FUNCTION OF GENES INVOLVED IN METABOLISM OF COMPLEX LIPIDS

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Abstract: Electrospray ionization tandem mass spectrometry in the precursor and neutral loss scanning modes is utilized to obtain profiles of the complex, polar lipids of plants. This method is rapid, accurate, and sensitive. The technique is being used to determine the metabolic functions of known genes, to implicate new metabolic pathways, and to help identify mutant genes from their functions.

Key Words: lipidomics; lipid profiling; electrospray ionization; mass spectrometry; phospholipids; galactolipids.

1 INTRODUCTION

Metabolomics may be viewed as a comprehensive strategy to study the function and levels of metabolites in relation to the function of genes and their proteins. In this context, lipidomics can be considered the branch of metabolomics in which non-water-soluble metabolites are studied.

The aims of our group's lipidomic strategies are to determine the role of gene products involved in lipid metabolism and to determine the importance of specific genes and specific lipid compositional changes in plant responses to stress and hormones. To determine the role. of gene products, mutants

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that lack the function of genes encoding lipid metabolic enzymes and putative lipid metabolic enzymes are being examined. Comparison of the metabolic responses of these mutants with the responses of wild-type plants allows identification of gene function, including identification of *in vivo* substrates and products of the gene products. Such comparisons can be made at the levels of specific tissues, cell types, or subcellular fractions. To understand the role of particular lipid compositional changes that may be brought about by particular genes, the lipid composition of mutant plants can be correlated with physiological responses to stress or hormones.

2 LIPID PROFILING METHODOLOGY

Traditional, chromatographic analysis of polar, complex lipids is a time-consuming process that involves separation of the lipids into classes, derivatization, and analysis of the fatty acyl chains. In contrast, mass spectrometry (MS)-based lipid analysis is a rapid process that produces a detailed profile of lipid molecular species. We are currently offering mass-spectrometry-based lipid analysis through the Kansas Lipidomics Research Center, which is described on the web at www.k-state.edu/lipid/lipidomics/. The lipid profiling process involves (1) solvent extraction of tissues from wild-type and/or mutant organisms, (2) addition of a mixture of phospho-lipid and/or galactolipid internal standards and appropriate solvents, and (3) analysis by electrospray ionization tandem mass spectrometry (ESI-MS/MS) (Brügger et al., 1997; Welti et al., 2002, 2003). This process produces spectra from which hundreds of lipid molecular species can be identified by head group and mass. The mass can be interpreted as the total number of acyl carbons and total number of acyl double bonds. A lipid profile can be obtained from a small amount of material, such as a few percent of an Arabidopsis leaf.

The lipid profiling methodology utilizes a tandem mass spectrometer ("triple quad" or MS/MS) with a collision cell, where fragmentation occurs, between the two mass spectrometers and a detector after the second mass spectrometer in the ion path. Our sample introduction is electrospray ionization (ESI). No preseparation is used. The sample is introduced by continuous infusion in solvent into the ESI source. Lipid molecular ions are produced from the lipid molecules. Phosphatidylcholine (PC) and phos-phatidylethanolamine (PE) are analyzed as singly charged positive $[M+H]^+$ ions, phosphatidylglycerol (PG), phosphatidylinositol (PI), phosphatidic acid (PA), and phosphatidylserine (PS) are analyzed as singly charged negative $[M–H]^–$ ions, and monogalactosyldiacylglycerol (MGDG) and digalacto-syldiacylglycerol (DGDG) are analyzed as singly charged positive $[M+Na]^+$ ions.

The lipidomic technology utilizes precursor and neutral loss scanning to allow detection of individual lipid molecular species in extracted biological samples that contain complex mixtures of non-water-soluble compounds that produce molecular ions with essentially every unit mass up to over 1,000 amu. Precursor and neutral loss scanning modes allow the user to obtain a separate spectrum for each class of polar lipids, while many other components are being simultaneously infused and ionized. To perform "precursor" scanning, the second mass spectrometer is set to allow only ions with a mass that corresponds to a charged fragment, characteristic of a particular head group class, to move to the detector. When scanning occurs in the first mass spectrometer, the second mass spectrometer effectively acts as a "filter", so that signal is recorded at the detector only when a molecular ion from the first mass spectrometer produces the characteristic head group fragment. Thus, the spectrum (mass of molecular ions scanned by the first mass spectrometer vs signal detected after the second mass spectrometer) that is collected shows only the lipid molecular ions of those species that can produce the head group fragment. Usually this corresponds to the lipids in one head group class. A neutral loss spectrum also depicts the lipids in a single class; neutral loss scanning is performed when the charge does not localize to the lipid head group fragment after fragmentation. This is the case with PE and PS. In these lipids, the fragment ions containing the two acyl species vary in mass as a function of the molecular ion acyl composition, but the *difference* in mass between the molecular ion and the charged diacyl-containing fragment is constant (corresponding to the mass of the neutral head group fragment). Thus, when the second mass spectrometer scans in synchrony with the first mass spectrometer with an offset that corresponds to the mass of the neutral head group fragment, signal at the detector is again observed only when the first mass spectrometer is at the mass of a molecular ion that generates the characteristic neutral loss of the head group.

In a lipid profiling experiment, a series of precursor and neutral loss scans are executed sequentially (Table 6-1). The signal for each molecular species is corrected for isotopic overlap of the lipid species with other species and then compared in magnitude with the signals of the internal standards (Welti et al., 2002). Currently, this methodology allows routine analysis of 144 polar plant lipid molecular species in eight head group classes. As mentioned, these species are identified in terms of total carbon number and total double bonds. More detailed information about the acyl species can be determined separately *via* product ion analysis of the molecular ions in the negative mode (Welti et al., 2002).

Table 6-1. Precursor and neutral loss scans for analysis of lipid species from plants

Time (min)	Scan mode	Fragment detected	Classes analyzed
3[a]	+	Precursor of 184^+	LysoPC/PC
2	+	Neutral loss of 141	LysoPE/PE
4	-	Precursor of 153^-	LysoPG/PG/PA
2	-	Precursor of 241^-	PI
3	-	Neutral loss of 87	PS
5	+	Precursor of 243^+	MGDG
5	+	Precursor of 243^+	DGDG

The first five scans are performed on an aliquot of extract dissolved in chloroform/ methanol/water (300:665:35) containing 10.5 mM ammonium acetate, while the MGDG and DGDG scans are performed on a second aliquot of extract that is dissolved in chloroform/methanol/water (300:665:35) containing 1.75 mM sodium acetate. Spectra are acquired sequentially by scanning in the listed modes for the indicated time period.

3 USES OF LIPID PROFILING TECHNOLOGY

Lipid profiling technology has been utilized to determine the metabolic functions of genes involved in lipid metabolism (Welti et al., 2002; Nandi et al., 2003, 2004; Abbadi et al., 2004; Li et al., 2004), to examine lipid changes during developmental processes (Fauconnier et al., 2003), to implicate new metabolic pathways (Welti et al., 2002), and to help identify mutant genes from their functions (Nandi et al., 2003). One example of determination of the metabolic function of a gene was for phospholipase Dα1, one of the 12 Arabidopsis gene products that encode phospholipase Ds. Lipid profiles of rosettes sampled before and after freezing stress from wild-type Arabidopsis were compared with similarly treated samples from Arabidopsis deficient in phospholipase Dα1. This comparison showed that phospholipase Dα1 accounts for about half of the PA formed upon freezing. These lipid profiles also showed that phospholipase Dα1 acts on PC, rather than PE or PG.

Lipid profiles of wild-type Arabidopsis during freezing suggested the existence of a previously undescribed pathway leading from MGDG to PA. During freezing, a molecular species of PA that is not detectable before freezing is formed. This species, 34:6 PA, is likely to be derived from MGDG, the only diacyl lipid class that contains large amounts of 34:6 diacylglycerol. The metabolic steps and the gene products involved in this pathway await elucidation.

An example of how lipid profiling expedited the identification and cloning of genes affecting biochemical processes *via* a candidate gene approach is provided by *SFD1*. Lipid profiling of the *ssi2 sfd1* mutant plants suggested

that the *SFD1* was involved in plastid lipid biosynthesis (Nandi et al., 2003). The profile suggested a lipid composition similar to that described by Miquel et al. (1998) for a mutant involved in glycerol phosphate metabolism. A survey of genes near the map location of *SFD1* identified a gene (At2g40690) that putatively encoded a DHAP reductase. Sequencing of *sfd1* mutant alleles confirmed that there were mutations in At2g40690. Finally, genetic complementation and studies of the SFD1 gene expressed in *Escherichia coli* confirmed the identity of *SFD1*.

4 LONG-TERM GOALS

The long-term goals of our group are to determine the roles of genes and enzymes that are involved and potentially involved in lipid metabolism in generating lipid compositional changes during plant stress. In addition, through the Kansas Lipidomics Research Center, we plan to continue to provide high-throughput, sensitive, and accurate lipid profiling and analysis. Finally, we will continue to develop mass-spectrometry-based lipid profiling strategies.

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