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Electrospray Ionization Multiple Stage Quadrupole Ion-Trap and Tandem Quadrupole Mass Spectrometric Studies on Phosphatidylglycerol from Arabidopsis leaves

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Abstract

Phosphatidylglycerol (PG) is the major phospholipid of plant chloroplasts. PG from *Arabidopsis thaliana* has an unusual fatty acyl chain, 3-*trans*-hexadecenoyl (Δ^3 16:1) in the *sn*-2 position of the major 18:3/ Δ^3 16:1-PG species, as well as in 18:2/ Δ^3 16:1-PG and 16:0/ Δ^3 16:1-PG. Upon low-energy collisionally activated dissociation (CAD) in a tandem quadrupole or in an ion-trap mass spectrometer, the $[M - H]^-$ ions of the PG molecules containing Δ^3 16:1 give product-ion spectra that are readily distinguishable from those arising from PGs without the Δ^3 16:1 species. The Δ^3 16:1-fatty acyl-containing PGs are characterized by MS² product-ion mass spectra that contain predominant $[M - H - 236]^-$ ions arising from loss of the Δ^3 16:1-fatty acyl substituent as a ketene. This is attributable to the fact that the α -hydrogen of the Δ^3 16:1-fatty acid substituent involved in the ketene loss is an allylic hydrogen, which is very labile. This leads to preferential neutral loss of 236 and drastic decline in the neutral loss of 254 (i.e., loss as a fatty acid), the unique features that signify the presence of Δ^3 16:1-fatty acyl containing PGs. The neutral loss scan of 236, thus provides a sensitive tandem quadrupole mass spectrometric means to identify Δ^3 16:1-containing PG species in lipid mixtures. This low-energy tandem mass spectrometric approach also permits the structures of the Arabidopsis PGs that consist of two isomeric structures to be unveiled.

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Keywords

Phosphatidylglycerol; plant chloroplast phosphatidylglycerol; 3-trans-hexadecenoic acid; tandem mass spectrometry; electrospray ionization

Introduction

Phosphatidylglycerol (PG) is the major phospholipid of plant chloroplasts and about 85% of PG in *Arabidopsis thaliana* leaves is localized in the chloroplast. PG is thought to play an important role in the ordered assembly and structural maintenance of the photosynthetic apparatus in thylakoid membranes [1–4]. It is required in photosystem II of photosynthesis [5,6]. The chloroplast membranes of all photosynthetic eukaryotes contain a high proportion of the unusual fatty 3-*trans*-hexadecenoic acid (Δ^3 16:1 or C16:1(3t)) which is always found esterified to the *sn*-2 of glycerol backbone of PG [7–10]. The Δ^3 16:1 acid is the product of the activity of a phosphatidylglycerol fatty acid desaturase, FAD4 (EC 1.14.99.*), which acts exclusively on fatty acyl chains in the *sn*-2 position of PG in plant plastids, probably in the thylakoid membranes [11–14]. Indeed, Δ^3 16:1 is believed to be found only in the photosynthetic membranes of eukaryotes [15].

The biological relevance of 3-*trans*-hexadecenoic acid in PG has been studied. For examples, loss of Δ^3 16:1 fatty acid in mutants of *Chlamydomonas reinhardtii* resulted in lack of proper assembly of the light-harvesting complexes associated with photosystem II [6,15]. Removal of Δ^3 16:1 fatty acid from PG by phospholipase-A2 treatment of isolated thylakoids was reported to alter the efficiency of light capture and to change the kinetics of fluorescence induction [16]. In addition, Chapman et al. reported that triazine-resistant plants have a higher level of PG-containing Δ^3 16:1 fatty acid in membrane fractions enriched in photosystem II [17]. Low temperature treatments also induce an increase in the relative content of both linolenic and 3-*trans*-hexadecenoic acids in thylakoid membrane PG of squash cotyledons [18].

The 3-*trans*-hexadecenoic acid is atypical because of the *trans* configuration, and because of the position of the double bond near the carboxyl (at C3) rather than the methyl end of the fatty acid. The 3-*trans*-hexadecenoic acid was also found in seaweed [19], in which the structure was established by GC as its fatty acid methyl ester, as well as by GC/MS of the dimethyl-oxazoline derivative [19,20]. Although structural studies on PG using tandem quadrupole mass spectrometry have been previously reported [21,22], plant PG species containing 3-*trans*-hexadecenoic acid at *sn*-2 have been identified at the true molecular species level only by a derivatization/HPLC method [23]. Herein, we describe the utilization of tandem quadrupole and multiple-stage quadrupole ion-trap mass spectrometry to discern the fragmentation mechanism underlying the unique pattern of product ion formation by PGs containing Δ^3 16:1. The presence of the Δ^3 16:1-fatty acid at the *sn*-2 position in PG results in a product-ion spectrum readily distinguishable from that obtained from PG that does not contain 3-*trans*-hexadecenoic acid.

Materials and Methods

Materials

Leaves of *Arabidopsis thaliana*, ecotype Columbia, were placed in 1 volume isopropanol with 0.01% butylated hydroxytoluene (as an anti-oxidant) at 75°C (approximately 1 leaf per mL isopropanol). After 15 min in hot isopropanol, 0.5 volumes chloroform and 0.2 volumes water were added. The tubes were agitated for 1 h, followed by removal of the extract. The plants were re-extracted with 1 volume chloroform:methanol (2:1) with 0.01 % butylated

hydroxytoluene 5 times with appropriately 30 min shaking each time. Solvent was evaporated from the combined extracts, and the residue was dissolved in chloroform. Activated silicic acid (Unisil, Clarkson Chemical Co., Williamsport, PA) was mixed with chloroform and packed into a column. The leaf extract was applied to the column and eluted in five fractions: Fraction I, chloroform:acetone (1:1, v/v; 5 column volumes); Fraction II, acetone (10 column volumes); Fraction III, chloroform:methanol (19:1, v/v; 10 column volumes); Fraction IV, chloroform:methanol (4:1, v/v; 10 column volumes); Fraction V, chloroform:methanol (1:1, v/v; 20 column volumes) [modified from 24]. Fraction V, which was enriched in PG, phosphatidylcholine and phosphatidylinositol, and also containing phosphatidylethanolamine and phosphatidylserine (results not shown), was used in all experiments. PG standards were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). All other chemicals were purchased from Fisher Scientific (Pittsburgh, PA).

Mass spectrometry

Low-energy CAD tandem mass spectrometry experiments were conducted both on a Finnigan (San Jose, CA) LCQ DECA ion-trap (IT) mass spectrometer (MS) with the Xcalibur operating system and on a Finnigan TSQ-7000 triple-stage quadrupole (TSQ) mass spectrometer with ICIS operating system. Fraction V of the plant lipid extract and methanolic PG standard solution were infused (3 $\mu\text{L}/\text{min}$) to the ESI source, where the skimmer was set at ground potential, the electrospray needle was set at 4.5 kV, and temperature of the heated capillary was 260°C. The automatic gain control of the ion trap was set to 5×10^7 , with a maximum injection time of 400 ms. Helium was used as the buffer and collision gas at a pressure of 0.75 mTorr. The MS^n experiments were carried out with a relative collision energy ranging from 30–40% and with an activation q value at 0.25. The activation time was set at 100 ms. Mass spectra were accumulated in the profile mode, typically for 3–5 min for MS^2 - and MS^3 -spectra. The mass resolution of the instrument was tuned to 0.6 Da at half peak height.

For product-ion spectra obtained with a TSQ instrument, the precursor ions were selected in the first quadrupole (Q1), collided with Ar (2.3 mTorr) in the rf-only second quadrupole (Q2) and analyzed in the third quadrupole (Q3). The collision energies were set at 32 eV. Both Q1 and Q3 were tuned to unit mass resolution and scanned at a rate of 3 sec/scan. The mass spectra were accumulated in the profile mode, typically for 5–10 min for a tandem mass spectrum.

Results and Discussion

The ESI full scan mass spectrum of the lipid extract obtained with a TSQ instrument in the negative-ion mode contains several deprotonated ($[\text{M} - \text{H}]^-$) PG species at m/z 719, 721, 741, 743, 745, 747 and 757 (Figure 1a). The spectrum is identical to that obtained with an IT instrument in the enhanced scan mode (zoom scan) (Figure 1b), which shows a low baseline and a well-resolved spectrum. The profile of the tandem mass spectrum obtained by neutral loss scan of 236 (Figure 1c) is also similar but this scan is selective, as will be discussed below. PG species consisting of two isomeric structures were observed (Table 1). The structural characterization and the mechanism(s) underlying the fragmentation processes under low-energy CAD are described below.

The fragmentation processes revealed by MS^n ($n=2,3$) tandem mass spectrometry

The formation of an ion corresponding to loss of a fatty acid substituent as a ketene (i.e., $[\text{M} - \text{H} - \text{R}'_x\text{CH}=\text{CO}]^-$) from a phospholipid is thought to be a charge-driven fragmentation process (CDF) involving participation of the α -hydrogen of the fatty acid substituent [15]. The anionic charge site at the phosphate abstracts the α -hydrogen of the fatty acid substituent, leading to the ketene loss (Scheme 1a). This fragmentation process probably is sterically more favorable with the fatty acid substituent at the *sn*-2 than with that at the *sn*-1 position, resulting

in the preferential formation of the ion at m/z 483 ($[M - H - R'_2CH=CO]^-$) over the ion at m/z 481 ($[M - H - R'_1CH=CO]^-$) in the MS² product-ion spectrum of the $[M - H]^-$ ion of 1-palmitoyl-2-palmitoleoyl-*sn*-glycero-3-phosphoglycerol (16:0/ Δ^9 16:1-PG) at m/z 719 (Figure 2a). The phosphate charge site also renders nucleophilic attack more favorable on C2 than C1 of the glycerol backbone (Scheme 1b), resulting in the m/z 253 ion ($R_2CO_2^-$) being more abundant than m/z 255 ($R_1CO_2^-$). These features in the product-ion spectra are readily applicable for determination of the fatty acid substituent identities and their positions on the glycerol backbone [22,25]. The spectrum also contains ions at m/z 465 and 463, arising from losses of the fatty acid substituents as acids. The former ion is more abundant than the latter, consistent with the notion that the fatty acyl species in the *sn*-2 position (Δ^9 16:1) is more readily lost.

The product-ion spectrum of the ion at m/z 719 from the lipid extract (Figure 2b) obtained with the same collision energy using a TSQ instrument is readily distinguishable from that arising from the 16:0/ Δ^9 16:1-PG standard (Figure 2a), consistent with the fact that the ion represents a 1-palmitoyl-2-(3-*trans*)-hexadecenoyl-*sn*-glycero-3-phosphoglycerol (16:0/ Δ^3 16:1-PG) [14]. Both the ions at m/z 253 and 255 are less prominent and the ion at m/z 253 is less abundant than the ion at m/z 255, a reversal in acyl anion abundances compared to those observed for 16:0/ Δ^9 16:1-PG. The ion at m/z 483 $[M - H - R'_2CH=CO]^-$, arising from loss of the Δ^3 16:1-fatty acid as a ketene is the most prominent. The dominance of the ion at m/z 483 is in accord with the decline of the ion at m/z 253, and consistent with the notion that the α -hydrogen of the fatty acid substituent participates in the ketene loss [15]. The α -hydrogen of the Δ^3 16:1-fatty acid substituent at *sn*-2 is situated between a carbonyl group and a carbon-carbon double bond. Thus, it is an allylic hydrogen and is very labile. Therefore, the fragmentation process leading to loss of the Δ^3 16:1-fatty acyl ketene (Scheme 1a) is the most facile pathway.

The CDF process leading to ketene loss (Scheme 1a) competes with the CDF process that leads to acyl anions by nucleophilic substitution (Scheme 1b). Because loss of the Δ^3 16:1-fatty acid at *sn*-2 as a ketene is the most facile pathway, the process leading to formation of $R_2CO_2^-$ at m/z 253 becomes less favorable. However, the process leading to m/z 481 due to loss of the 16:0-fatty acid substituent at *sn*-1 as a ketene (Scheme 1a) is less facile than that leading to the $R_1CO_2^-$ anion at m/z 255 (Scheme 1b), attributable to the fact that the α -hydrogen of the 16:0-fatty acid is less labile. The very facile ketene loss of the *sn*-2 substituent in the PG containing *sn*-2 Δ^3 16:1 leads to a higher ratio of the $R_1CO_2^-$ ion to the $R_2CO_2^-$ ion than observed for phospholipids not containing an allylic α -hydrogen in the *sn*-2 fatty acid (Figure 2b vs. 2a).

The MS² product-ion spectra of 16:0/ Δ^9 16:1-PG (Figure 2c) and 16:0/ Δ^3 16:1-PG (Figure 2d) obtained with an IT instrument are similar to those obtained with a TSQ. However, the carboxylate anions at m/z 253 and 255 are of low abundance, and the ions at m/z 463 and 465 arising from losses of the fatty acid substituents are more abundant, consistent with the notion that the fragmentation processes in an ion-trap are initiated by resonance excitation so that consecutive fragmentations induced by multiple collisions, as seen in an TSQ instrument, are minimal.

The ions at m/z 465 (719 - R_2CO_2H) and 463 (719 - R_1CO_2H) gives rise to ions at 391 and 389, respectively, by loss of a $C_3H_6O_2$ residue. The fragmentation processes are supported by the IT MS³-spectra of the m/z 465 (719 \rightarrow 465) (Figure 2e) and 463 (719 \rightarrow 463) ions (not shown), which are dominated by the ions at m/z 391 (Figure 2e) and 389, respectively. The ions at m/z 391 (483 - 92) and 389 (481 - 92) can also arise from m/z 483 and 481, respectively, by elimination of a glycerol residue. However, these fragmentation pathways are less favorable, as revealed by the IT MS³-spectra of m/z 483 (719 \rightarrow 483) (not shown). The ions at m/z 391 and 389 also give rise to the ions at m/z 255 and 253, respectively, by neutral loss of 136 [22, 25,26]. These data suggest that, in the TSQ product-ion spectra, the carboxylate anions at m/z

m/z 255 and 253 of 16:0/ Δ^9 16:1-PG (Figure 2a) and 16:0/ Δ^3 16:1-PG (Figure 2b) can arise from consecutive fragmentations in addition to the nucleophilic substitution pathway shown in Scheme 1b. This is consistent with the low abundance of the carboxylate anions and the higher abundance of the ions at m/z 465/463 and 391/389 in the IT MS² product-ion spectra (Figure 2c and 2d).

The ion at m/z 741 (Figure 1) undergoes fragmentation processes similar to 16:0/ Δ^3 16:1-PG and gives rise to a product-ion spectrum similar to that shown in Figure 2b. The spectrum (Figure 2f) is dominated by the ion at m/z 505 (741 – 236) corresponding to loss of a 16:1-fatty acid substituent as a ketene and analogous to the m/z 483 ion in Figure 2b. The profile of the MS²-spectrum obtained with an IT instrument (data not shown) is similar to that shown in Figure 2d. The results indicate that the compound consists of a Δ^3 16:1-fatty acid substituent, most likely at *sn*-2. The ions at m/z 481, arising from loss of 18:3-fatty acid as a ketene and at m/z 277, corresponding to an 18:3-fatty acid anion are also present in the spectrum, suggesting that an 18:3 fatty acid substituent resides at *sn*-1. The spectrum also contains ions at m/z 227, 209, 171 and 153, ions commonly observed for PG [15], confirming that the ion represents 18:3/ Δ^3 16:1-PG.

Characterization of PG consisting of isomeric structures

Product-ion analysis revealed two isomeric structures for the ions at m/z 743, 745 and 747 (Table 1). Determination of the structures is exemplified by the ion at m/z 743. The MS² product-ion spectrum obtained with a TSQ instrument (Figure 3a) contains ions at m/z 227, 209, 171 and 153 that are diagnostic ions for PG, along with two pairs of the carboxylate anions at m/z 255/277 and 279/253, indicating that the ion may consist of 18:3/16:0-PG and 18:2/16:1-PG isomers. The 18:3/16:0-PG structure is recognized by the observation of the ions at m/z 505 and 487, arising from loss of a 16:0-fatty acid substituent as a ketene and as an acid respectively, and the ions at m/z 483 and 465, arising from the analogous losses of an 18:3-fatty acid substituent. The ion at m/z 505 is more abundant than m/z 483, and the ion at m/z 487 is also more abundant than the ion at m/z 465, indicating that the 16:0- and 18:3-fatty acid substituents are located at *sn*-2 and *sn*-1, respectively, consistent with the observation of a greater abundance of the ion at m/z 255 than the ion at m/z 277. The structural assignment is further supported by the MS² product-ion spectrum obtained with an IT MS (Figure 3b), in which the differential formation of the above fragment ions also can be seen. The spectrum also features the greater prominence of the ion at m/z 413 (487 – 74), arising from loss of C₃H₆O₂ from m/z 487, as compared to the ion at m/z 391 (465 – 74), arising from further loss of C₃H₆O₂ from m/z 465.

The assignment of the 18:2/ Δ^3 16:1-PG structure is supported by the presence of the ion at m/z 507 (Figures 3a and 3b), corresponding to loss of the 16:1-fatty acid substituent as a ketene. This ion is most prominent in the product-ion spectrum obtained with an ion-trap (Figure 3b), but the ion at m/z 489, arising from loss of the 16:1-fatty acid substituent as an acid, is of low abundance, suggesting that the 16:1-fatty acid substituent is 3-*trans*-hexadecenoic acid, the unusual fatty acid attached to the *sn*-2 position of PG found in plant chloroplasts. The assignment of the 18:2/ Δ^3 16:1-PG structure is also consistent with the observation of a higher abundance of the ion at m/z 279 than the ion at m/z 253, similar to the profile seen for the 16:0/ Δ^3 16:1-PG (Figure 2b) and 18:3/ Δ^3 16:1-PG (Figure 2f), which contain the Δ^3 16:1-fatty acid substituent at *sn*-2.

Identification of PG species containing Δ^3 16:1-fatty acid substituent via constant neutral loss (CNL) scanning

As seen earlier, the product-ion spectra of PG containing the Δ^3 16:1-fatty acid substituent at *sn*-2 are dominated by the [M – H – 236][–] ion, arising from neutral loss of the Δ^3 16:1-fatty

acyl substituent as a ketene, and the $[M - H - 254]^-$ ion arising from neutral loss of the $\Delta^3 16:1$ -fatty acyl substituent as an acid is of low abundance. As shown in Figure 1c, the spectrum acquired from CNL scanning of 236 of the lipid extract is similar to the full scan (Figures 1a and 1b), but only peaks from PGs that consist of $\Delta^3 16:1$ -fatty acid are observed. The spectrum clearly demonstrates that the PG species from plant chloroplasts mainly contain a $\Delta^3 16:1$ -fatty acid substituent, consistent with the results from the structural analysis of the individual ions from product-ion spectra (Table 1). In contrast, the signals of the similar ions in the spectrum acquired from CNL scanning of 254 (data not shown) are weak and the intensity of the ion at m/z 741, for example, is <2% than that seen in Figure 1c. This drastic change in the relative sensitivity between the spectra obtained from CNL scanings of 236 and of 254 provides distinction of the $\Delta^3 16:1$ -fatty acid bearing PG from, for example, $\Delta^9 16:1$ -bearing PGs, which also undergo similar neutral losses (i.e. CNL of 254 and 236), but in a different manner. Therefore, tandem quadrupole mass spectrometry with CNL scanning of 236, in combination with CNL scanning of 254 that confirms that the species indeed contains $\Delta^3 16:1$ -fatty acid, provides a useful means for sensitive detection of PG species containing a $\Delta^3 16:1$ -fatty acid substituent in mixtures.

In Figure 1c, an ion at m/z 757 is also present, suggesting that the ion may arise from a PG containing a $\Delta^3 16:1$ -fatty acid substituent, probably a hydroxylated 18:3/ $\Delta^3 16:1$ -PG. This structural assignment is based on the finding that the profile of the IT MS²-spectrum of the ion at m/z 757 (Figure 3c), is similar to that shown in Figure 2d, supporting the presence of a $\Delta^3 16:1$ -fatty acid substituent. The ion at m/z 293 may represent a hydroxylated 18:3-fatty acid (h18:3) substituent. This speculation is based on the notion that the ion at m/z 275 ($293 - H_2O$), arising from loss of H_2O is the predominant ion in the IT MS³-spectrum of 293 ($757 \rightarrow 293$) (Figure 3d) [27]. The presence of the ion at m/z 429 ($503 - 74$), arising from m/z 503 by loss $C_3H_6O_2$ and the presence of the ion at m/z 739, arising from a water loss from 757 (Figure 3c) are also consistent with the presence of a hydroxylated 18:3-fatty acid residue in the molecule. However, further study is necessary to support the assigned structure and to confirm that the compound is indeed in the PG mixture.

Conclusions

The fragmentation processes observed for the PG species bearing a $\Delta^3 16:1$ -fatty acid at *sn*-2 are consistent with the mechanisms previously proposed by us [22,25,26]. These PG species are readily distinguishable from other species in this phospholipid class by their unique low-energy tandem mass spectra from the $[M - H]^-$ ion, in which the fragment ion reflecting loss of the $\Delta^3 16:1$ -fatty acid as a ketene (i.e., the $[M - H - 236]^-$) is prominent due to the labile nature of the α -hydrogen of $\Delta^3 16:1$ -fatty acid substituent. Recently, Moe et al. [28] and Thomas et al. [29] described ESI tandem mass spectrometric methods for location of the olefinic sites of phospholipids, following conversion to their 1,2-di-hydroxy derivatives by OsO_4 [28] or on-line ozonolysis of double bonds to yield both a terminal oxoalkanoyl and a terminal C-hydroperoxy, C-methoxy alkanoyl radical containing phospholipid anions [29]. These approaches are straightforward and should be useful, in particular, for locating the double bond (s) of the fatty acid substituents from various isomers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Sakurai I, Hagio M, Gombos Z, Tyystjarvi T, Paakkari V, Aro EM, Wada H. Requirement of Phosphatidylglycerol for Maintenance of Photosynthetic Machinery. *Plant Physiol* 2003;133:1376–1384. [PubMed: 14551333]
2. Sato N. Roles of the Acidic Lipids Sulfoquinovosyl Diacylglycerol and Phosphatidylglycerol in Photosynthesis: Their Specificity and Evolution. *J Plant Res* 2004;117:495–505. [PubMed: 15538651]
3. Frentzen M. Phosphatidylglycerol and Sulfoquinovosyldiacylglycerol: Anionic Membrane Lipids and Phosphate Regulation. *Curr Opin Plant Biol* 2004;7:270–276. [PubMed: 15134747]
4. Joyard J, Maréchal E, Miège C, Block MA, Dorne A, Douce R. Structure, distribution and biosynthesis of glycerolipids from higher plant chloroplasts. In: Siegenthaler PA, Murata N, editors. *Lipids in Photosynthesis: Structure, Function and Genetics*. Kluwer Academic Publishers; Dordrecht, the Netherlands: 1998. p. 21-52.
5. Hagio M, Gombos Z, Várkonyi Z, Masamoto K, Sato N, Tsuzuki M, Wada H. Direct Evidence for Requirement of Phosphatidylglycerol in Photosystem II of Photosynthesis. *Plant Physiol* 2000;124:795–804. [PubMed: 11027727]
6. Pineau B, Girard-Bascou J, Eberhard S, Choquet Y, Tremolieres A, Gerard-Hirne C, Bennardo-Connan A, Decottignies P, Gillet S, Wollman FA. A Single Mutation that Causes Phosphatidylglycerol Deficiency Impairs Synthesis of Photosystem II Cores in *Chlamydomonas reinhardtii*. *Eur J Biochem* 2004;271:329–338. [PubMed: 14717700]
7. Haverkate F, de Gier J van Deenen LL. The occurrence of delta 3-trans-hexadecenoic acid in phosphatidyl glycerol from spinach leaves. *Experientia* 1964;20:511–512. [PubMed: 5856341]
8. Dubacq JP, Trémolières A. Occurrence and function of phosphatidylglycerol containing Δ^3 -trans-hexadecenoic acid in photosynthetic lamellae. *Physiol Vég* 1983;21:293–312.
9. Selstam E. Development of thylakoid membranes with respect to lipids. In: Siegenthaler P-A, Murata N, editors. *Advances in Photosynthesis, Vol 6, Lipids in Photosynthesis: Structure, Function and Genetics*. Kluwer Academic Publishers; Dordrecht: 1998. p. 209-224.
10. Browse J, Warwick N, Somerville CR, Slack CR. Fluxes through the prokaryotic and eukaryotic pathways of lipid synthesis in the '16:3' plant *Arabidopsis thaliana*. *Biochem J* 1986;235:25–31. [PubMed: 3741384]
11. Roughan PG. Phosphatidyl choline: Donor of 18-carbon Unsaturated Fatty Acids for Glycerolipid Biosynthesis. *Lipids* 1975;10:609–614. [PubMed: 1186446]
12. Mackender RO, Leech RM. The Galactolipid, Phospholipid, and Fatty Acid Composition of the Chloroplast Envelope Membranes of *Vicia faba*. L. *Plant Physiol* 1974;53:496–502. [PubMed: 16658731]
13. Ohnishi M, Thompson GA Jr. Biosynthesis of the Unique trans-Delta 3-Hexadecenoic Acid Component of Chloroplast Phosphatidylglycerol: Evidence Concerning Its Site and Mechanism of Formation. *Arch Biochem Biophys* 1991;288:591–599. [PubMed: 1898051]
14. Beisson F, Koo AJK, Ruuska S, Schwender J, Pollard M, Thelen J, Paddock T, Salas J, Savage L, Milcamps A, Mhaske VB, Cho Y, Ohlrogge JB. *Arabidopsis thaliana* Genes Involved in Acyl Lipid Metabolism. A 2003 Census of the Candidates, a Study of the Distribution of Expressed Sequence Tags in Organs, and a Web-Based Database; *Plant Physiol*. 2003. p. 681-697. URL: <http://www.plantbiology.msu.edu/lipids/genesurvey/index.htm>
15. Dubertret G, Mirshahi A, Mirshahi M, Gerard-Hirne C, Tremolieres A. Evidence from in Vivo Manipulations of Lipid Composition in Mutants that the Delta 3-trans-Hexadecenoic Acid-Containing Phosphatidylglycerol Is Involved in the Biogenesis of the Light-Harvesting Chlorophyll A/B-Protein Complex of *Chlamydomonas reinhardtii*. *Eur J Biochem* 1994;226:473–482. [PubMed: 8001565]
16. Krupa Z. The action of lipases on chloroplast membranes. III. The effect of lipid hydrolysis on chlorophyll-protein complexes in thylakoid membranes. *Photosynthesis Res* 1984;5:177–184.

17. Chapman DJ, De-Felice J, Barber J. Characteristics of Chloroplast Thylakoid Lipid Composition Associated with Resistance to Triazine Herbicides. *Planta* 1985;166:280–285.
18. Xu Y, Siegenthaler PA. Low Temperature Treatments Induce an Increase in the Relative Content of Both Linolenic and Δ^3 -trans-Hexadecenoic Acids in Thylakoid Membrane Phosphatidylglycerol of Squash Cotyledons. *Plant Cell Physiol* 1997;38:611–618.
19. Lamberto M, Ackman RG. Confirmation by gas chromatography/mass spectrometry of two unusual trans-3-monoethylenic fatty acids from the Nova Scotian seaweeds *Palmaria palmata* and *Chondrus crispus*. *Lipids* 1994;29:441–444. [PubMed: 8090067]
20. Lamberto M, Ackman RG. Positional isomerization of trans-3-hexadecenoic acid employing 2-amino-2-methyl-propanol as a derivatizing agent for ethylenic bond location by gas chromatography/mass spectrometry. *Anal Biochem* 1995;230:224–228. [PubMed: 7503411]
21. Welti R, Wang X, Williams TD. Electrospray Ionization Tandem Mass Spectrometry Scan Modes for Plant Chloroplast Lipids. *Anal Biochem* 2003;314:149–152. [PubMed: 12633615]
22. Hsu FF, Turk J. Studies on Phosphatidylglycerol with Triple Quadrupole Tandem Mass Spectrometry with Electrospray Ionization: Fragmentation Processes and Structural Characterization. *J Am Soc Mass Spectrom* 2001;12:1036–1043.
23. Xu Y, Siegenthaler PA. Phosphatidylglycerol Molecular Species of Photosynthetic Membranes Analyzed by High-Performance Liquid Chromatography: Theoretical Considerations. *Lipids* 1996;31:223–229. [PubMed: 8835412]
24. Christie, WW. *Lipid Analysis*. Vol. 2. Pergamon Press; Oxford, England: 1982. p. 109-110.
25. Hsu, FF.; Turk, J. Electrospray Ionization with Low-Energy Collisionally Activated Dissociation Tandem Mass Spectrometry of Complex Lipids: Structural Characterization and Mechanisms of Fragmentation. In: Byrdwell, WC., editor. *Modern Methods for Lipid Analysis by Liquid Chromatography/Mass Spectrometry*. AOCS Press; Champaign, IL: 2005. p. 61-178.
26. Hsu FF, Turk J. Charge-driven Fragmentation Processes in Diacyl Glycerophosphatidic Acids upon Low-Energy Collisional Activation. A Mechanistic Proposal. *J Am Soc Mass Spectrom* 2000;11:797–803. [PubMed: 10976887]
27. Hong S, Lu Y, Yanag R, Gotlinger KH, Petasis NP, Serhan CN. Resolvin D1, Protectin D1, and Related Docosahexaenoic Acid-Derived Products: Analysis via Electrospray/Low Energy Tandem Mass Spectrometry Based on Spectra and Fragmentation Mechanisms. *J Am Soc Mass Spectrom*. 2006In press
28. Moe MK, Anderssen T, Strøm MB, Jensen E. Total structure characterization of unsaturated acidic phospholipids provided by vicinal di-hydroxylation of fatty acid double bonds and negative electrospray ionization mass spectrometry. *J Am Soc Mass Spectrom* 2005;16:46–59. [PubMed: 15653363]
29. Thomas MC, Mitchell TW, Blanksby SJ. Ozonolysis of Phospholipid Double Bonds during Electrospray Ionization: A New Tool for Structure Determination. *J Am Chem Soc* 2006;128:58–59. [PubMed: 16390120]

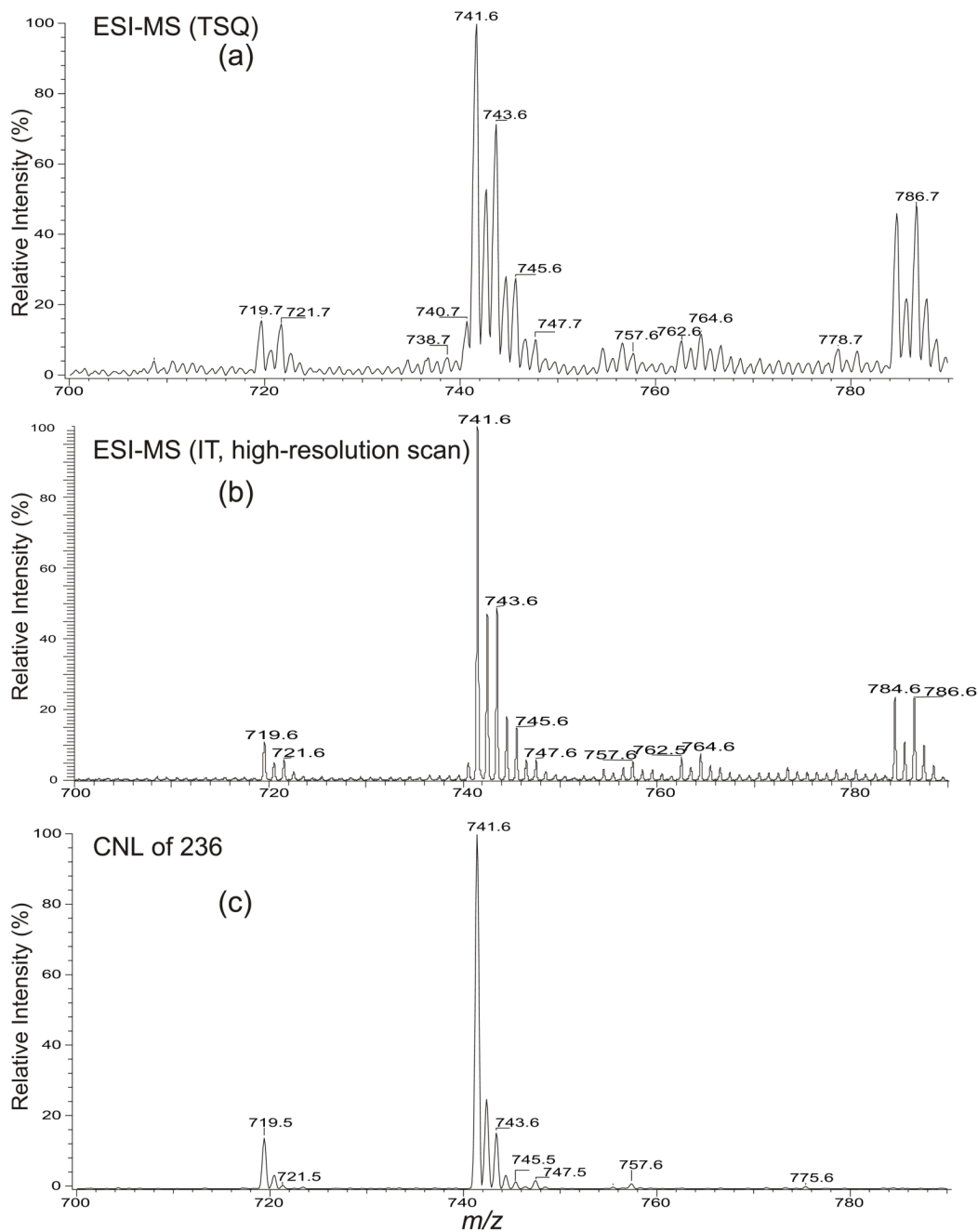


Figure 1.

The full scan mass spectra of the lipid extract from plant chloroplasts obtained with (a) a tandem quadrupole, and (b) an IT instrument. Panel (c) shows the tandem mass spectrum of the same mixture acquired by CNL of 236, exhibiting the PG species that contain a 16:1-fatty acid substituent, mainly a Δ^3 16:1-fatty acid substituent at *sn*-2 in the mixture.

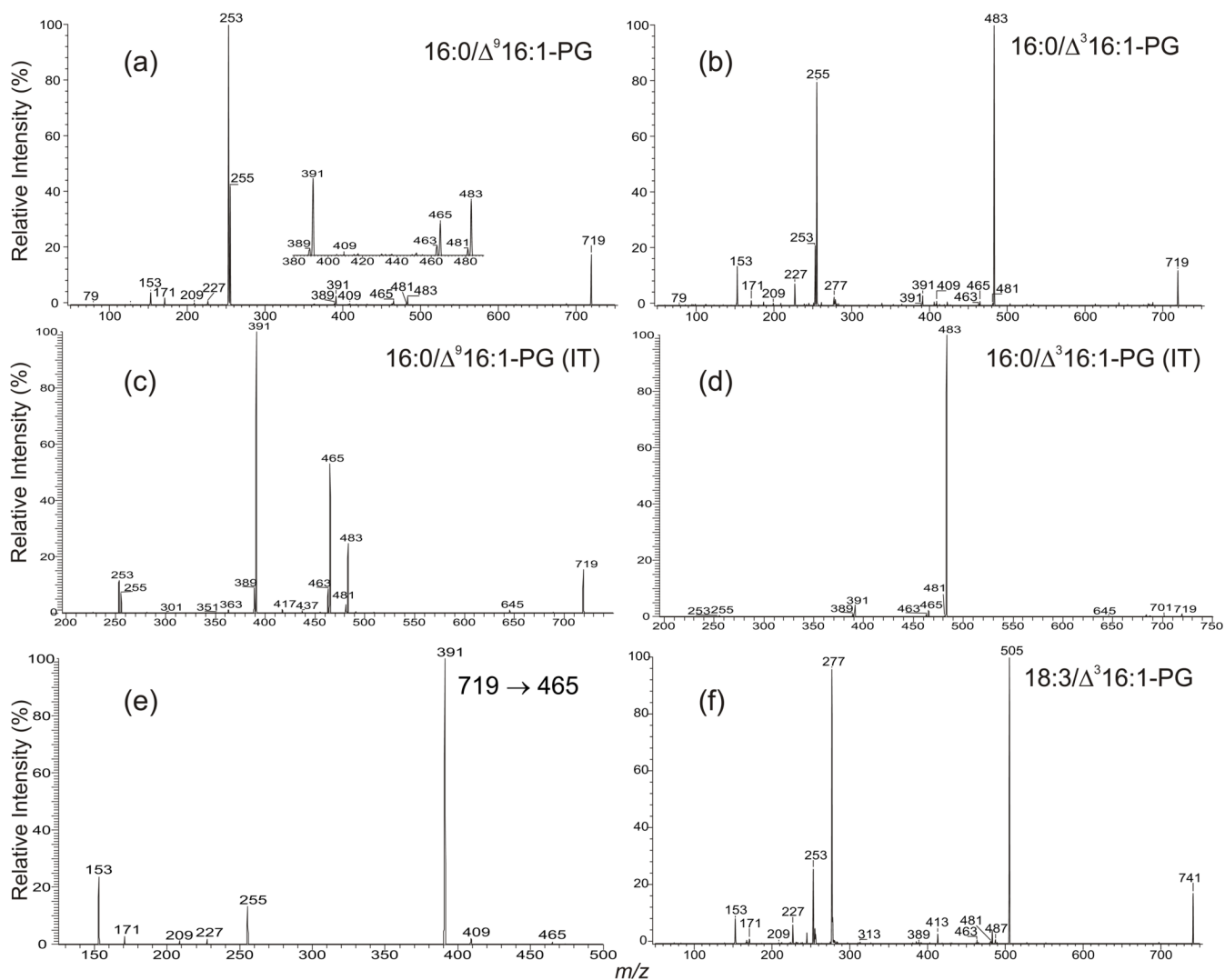


Figure 2.

The TSQ product-ion spectra of (a) 16:0/Δ⁹16:1-PG, (b) 16:0/Δ³16:1-PG, and the corresponding IT MS² spectra of (c) 16:0/Δ⁹16:1-PG, (d) 16:0/Δ³16:1-PG. The MS³-spectrum of the ion at *m/z* 465 (719 → 465) (e) illustrates that the ions at *m/z* 227, 171 and 153 that are characteristic ions of PG and the ion at *m/z* 391, which is a precursor ion of the carboxylate anion at *m/z* 255 arise from sequential fragmentation of *m/z* 465. Panel (f) is the product-ion spectrum of the ion at *m/z* 741, which is similar to the spectrum shown in Panel b, suggesting the presence of 18:3/Δ³16:1-PG, the major species found in the PG extract.

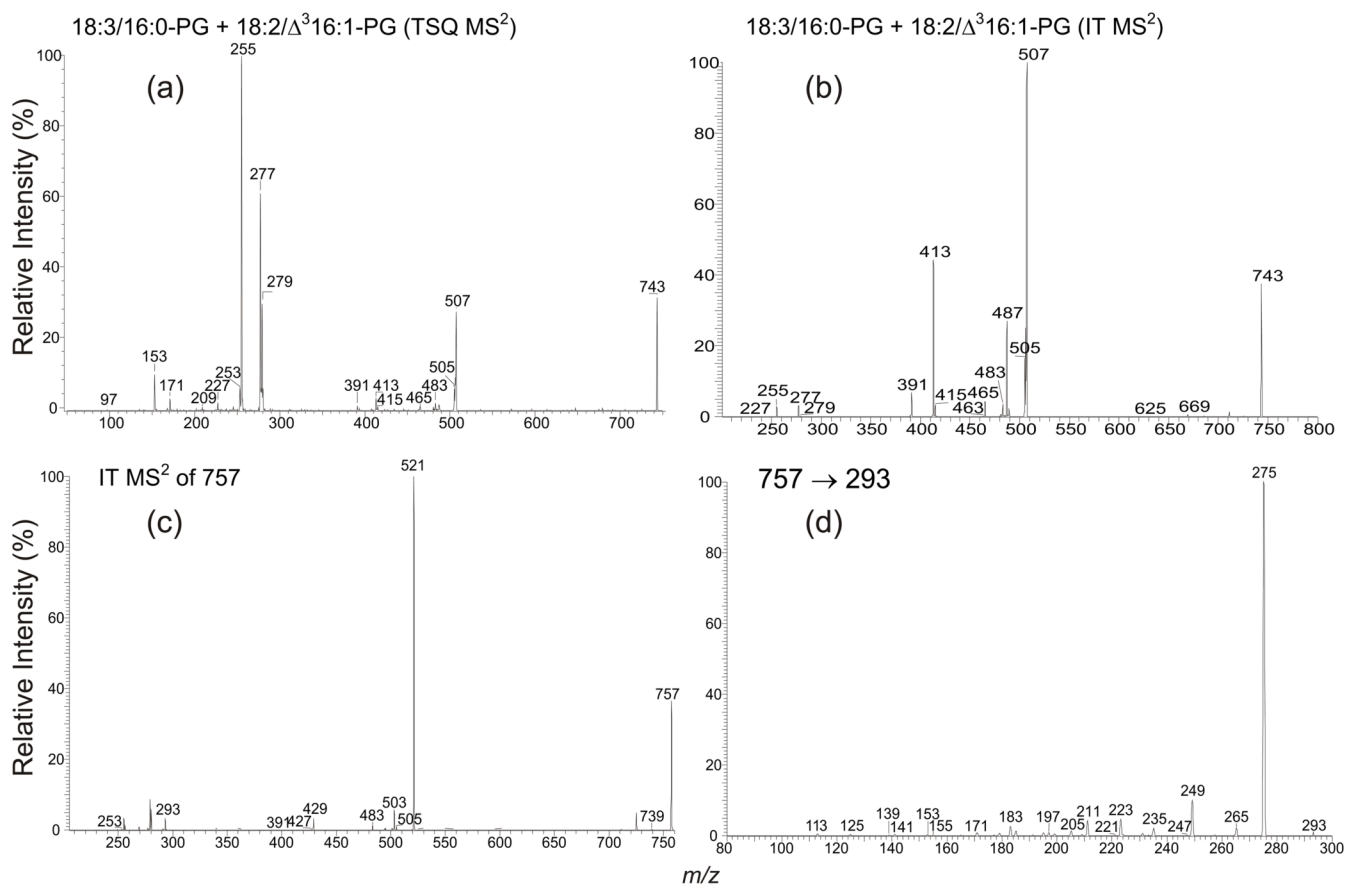
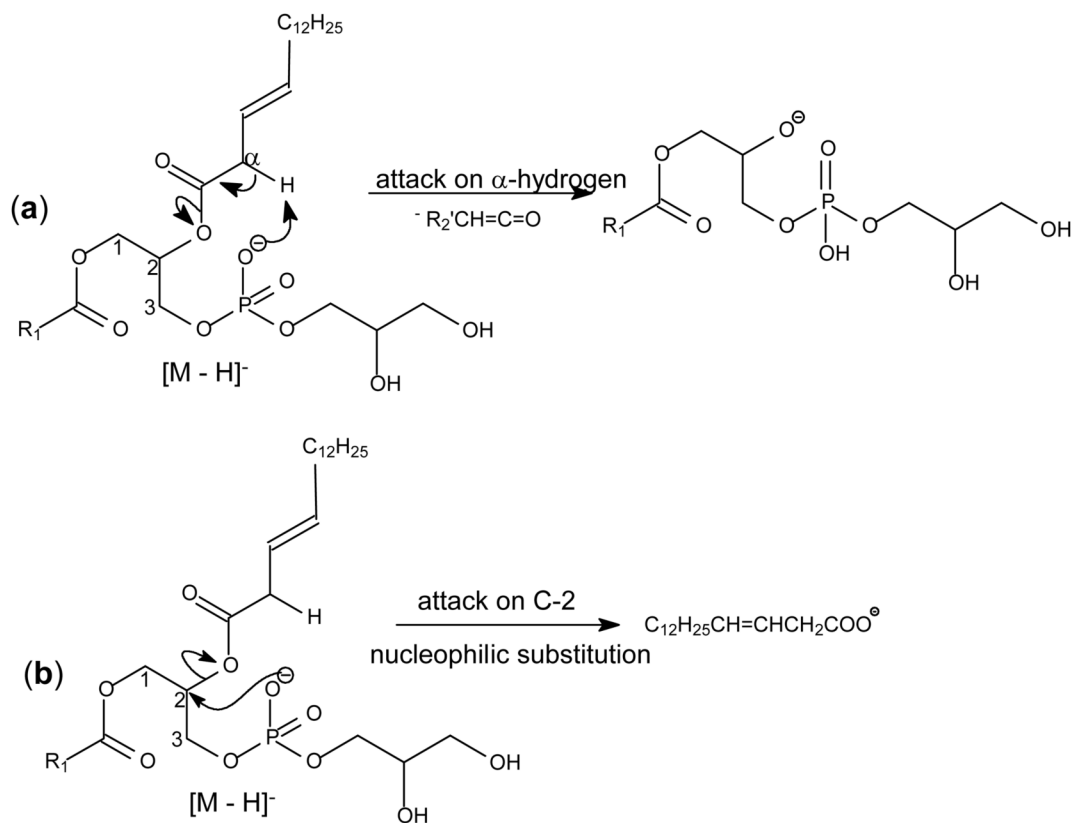


Figure 3.

The product-ion spectrum of the ion at m/z 743 obtained with (a) a TSQ, and (b) an IT instrument. Panel (c) is the IT MS²-spectrum of the ion at m/z 757, which may consist of a h18:3/ Δ^3 16:1-PG structure. The presence of the hydroxylated 18:3-fatty acid substituent is consistent with the presence of a prominent ion at m/z 275, corresponding to loss of H₂O from m/z 293, as shown in the MS³-spectrum of m/z 293 (757 \rightarrow 293) (d).

**Scheme 1.**

The CDF processes leading to (A) ketene loss, and (B) formation of carboxylate anion. The schemes show the fragmentation processes occurred at sn-2, which are sterically more favorable.

Table 1

Composition of PG from plant chloroplasts.*

[M – H] ⁻	Structure	Relative abundance (% of base peak)
719.6	16:0/ Δ^3 16:1-PG	15
721.6	16:0/16:0-PG	5
741.6	18:3/ Δ^3 16:1)-PG	100
743.6	18:2/ Δ^3 16:1-PG & 18:3/16:0-PG	50
745.6	18:2/16:0-PG & 18:1/ Δ^3 16:1-PG	10
747.6	18:1/16:0-PG & 18:0/ Δ^3 16:1-PG	8
757.6	** h18:3/ Δ^3 16:1-PG	6

* Data are derived from product-ion analysis with IT and TSQ mass spectrometers.

** h denotes hydroxylated