Positional Analysis of Fatty Acids in Phospholipids by PLA_2 Treatment

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[Abstract] Plant phospholipids can be produced in the endoplasmic reticulum or plastids. Lipids from different sources can be distinguished by the fatty acid profile, in terms of the preferred fatty acid species esterified to the *sn-1* or *sn-2* position of the glycerol backbone (Ohlrogge and Browse, 1995). This protocol is used to determine the fatty acid profile in total plant phospholipids by the treatment of *sn-2* specific phospholipase A_2 (PLA_2).

Materials and Reagents

1. Glass tubes [PYREX™ screw cap culture tubes with PTFE lined phenolic caps (Thermo Fisher Scientific, Fisher Scientific™, catalog number: 14-932H)]
2. Pipette 100-1,000 µl (Eppendorf Research 2100)
3. Axygen™ 1,000 µl universal pipette tips (Thermo Fisher Scientific, Fisher Scientific™, catalog number: 14-222-690)
4. Total plant lipids
   Note: Extracted by the method described in the companion lipid extraction and profiling protocol by the same authors (Liu and Wang, 2016) and the original publication (Welti et al., 2002)
5. Chloroform (Thermo Fisher Scientific, catalog number: C607)
6. PLA_2 from honey bee venom (Sigma-Aldrich, catalog number: P9279)
   Note: Dissolved in reaction buffer to 1 unit/µl before use.
7. Sodium chloride (NaCl) (Sigma-Aldrich, catalog number: S7653)
8. Nitrogen gas
9. HEPES (Sigma-Aldrich, catalog number: H3375)
10. Calcium chloride ($CaCl_2$) (Sigma-Aldrich, catalog number: C8106)
11. Magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$) (Sigma-Aldrich, catalog number: M2670)
12. Methanol (Thermo Fisher Scientific, Fisher Scientific™, catalog number: A456)
13. Reaction buffer (see Recipes)
14. NaCl solution (see Recipes)
15. Extraction solution (see Recipes)

Equipment

1. Nitrogen evaporator N-EVAP (Meyer Organomation, model: 111)
2. Ultrasonic processor with probe, work at 30% power (GEX 130PB) or similar equipment [*e.g.*, ultrasonic processor, (Cole-parmer, model: EW-04714-51)]
3. Incubator (Thermo Fisher Scientific, model: Isotemp 537D) or similar equipment [*e.g.*, microbiological incubators (Thermo Fisher Scientific, Isotemp™, catalog number: 15-103-0513)]

Procedure

1. Total plant lipids were reconstituted by chloroform and the amount of lipids corresponding to 0.2 mg dry weight were aliquoted in glass tubes. Same amount of untreated lipid samples will be used as controls in step 9.
2. Lipids were dried in the nitrogen evaporator thoroughly (>30 min), mild heating (<50 °C) may be used to accelerate the process.
3. Dried lipids were hydrated in 500 µl reaction buffer at room temperature for >10 min, followed by sonication (30% power, on ice) to disperse the lipids uniformly.
4. 10 units (10 µl) of PLA_2 were added to the reaction, mixed by vortex. The reaction mixtures were incubated at 37 °C for 2 h to completely digest phospholipids.
5. 500 µl of 500 mM NaCl was added to the mixture to facilitate phase separation. 2 ml extraction solution was added and vortexed to terminate the reaction and extract lipids.
6. Centrifuge at ~200 *x g* for 10 min. Transfer the bottom (organic) phase to another glass tube.
7. Add 1 ml chloroform and repeat step 6, twice for complete extraction.
8. Dry extracted lipids under nitrogen gas, reconstitute the lipids in 50 µl chloroform.
9. Use total lipids corresponding to 0.2 mg dry weight as control, profile both digested and undigested lipids in mass spectrometry as described in the companion lipid extraction and profiling protocol by the same authors (Liu and Wang, 2016). Three or more biological repeats are recommended and Student's *t*-test is used for statistical analysis. Compare regular lipids in undigested samples and lysolipids in digested samples to determine position/composition of fatty acid in different lipid classes. For more quantitative results and more phospholipids classes besides phosphatidylcholine (PC) and phosphatidylethanolamine (PE), lipid samples can first be resolved by thin layer chromatography (TLC) (Refer to the method in Liu *et al.,* 2015) and each lipid/lysolipid spot can be quantified by transmethylation and GC analysis (Politz *et al.,* 2013).

Representative data

Representative data from Liu *et al.*, 2015 (Figure 1). Total lipids were digested by PLA_2, followed by analysis of PC and LysoPC by mass spectrometry. The increase of LPC16 in digested sample and increase of PC34 in undigested samples in RNAi lines compared with WT are similar, suggesting the increased PC34 in RNAi lines have C16 fatty acid esterified to the *sn-1* position, which originates from the eukaryotic pathway.

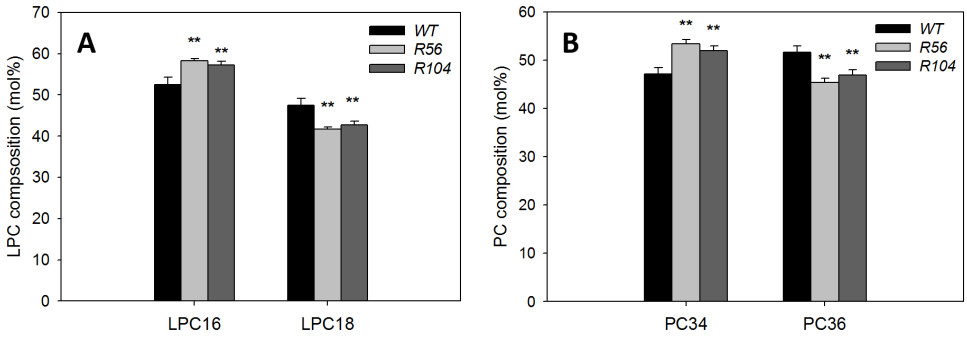

Figure 1. Analysis of fatty acyl chain position/composition in phosphatidylcholine (PC). A. Content of LysoPC with C16 and C18 fatty acids after PLA_2 treatments of lipids from two *AAPT* RNAi lines and WT plants. B. Content of PC with C34 and C36 fatty acids without PLA_2 treatments of lipids from two *AAPT* RNAi lines and WT plants. * and ** mark differences between WT and mutant at $P < 0.05$ and at $P < 0.01$, respectively based on Student's *t*-test. Values are means ± SD (n = 3).

Recipes

1. Reaction buffer
   100 mM HEPES, pH 7.4
   10 mM $CaCl_2$
   10 mM $MgCl_2$
   Note: Stable in room temperature.
2. NaCl solution
   500 mM NaCl
   Note: Stable in room temperature.
3. Extraction solution
   66.7% (v/v) Chloroform
   33.3% (v/v) Methanol
   Note: made before use

Acknowledgments

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