Video Article

A Quantitative Assessment of The Yeast Lipidome using Electrospray Ionization Mass Spectrometry

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Abstract

Lipids are one of the major classes of biomolecules and play important roles membrane dynamics, energy storage, and signalling¹⁻⁴. The budding yeast *Saccharomyces cerevisiae*, a genetically and biochemically manipulable unicellular eukaryote with annotated genome and very simple lipidome, is a valuable model for studying biological functions of various lipid species in multicellular eukaryotes^{2,3,5}. *S. cerevisiae* has 10 major classes of lipids with chain lengths mainly of 16 or 18 carbon atoms and either zero or one degree of unsaturation^{6,7}. Existing methods for lipid identification and quantification - such as high performance liquid chromatography, thin-layer chromatography, fluorescence microscopy, and gas chromatography followed by MS - are well established but have low sensitivity, insufficiently separate various molecular forms of lipids, require lipid derivatization prior to analysis, or can be quite time consuming. Here we present a detailed description of our experimental approach to solve these inherent limitations by using survey-scan ESI/MS for the identification and quantification of the entire complement of lipids in yeast cells. The described method does not require chromatographic separation of complex lipid mixtures recovered from yeast cells, thereby greatly accelerating the process of data acquisition. This method enables lipid identification and quantification at the concentrations as low as g/ml and has been successfully applied to assessing lipidomes of whole yeast cells and their purified organelles. Lipids extraction from whole yeast cells for using this method of lipid analysis takes two to three hours. It takes only five to ten minutes to run each sample of extracted and dried lipids on a Q-TOF mass spectrometer equipped with a nano-electrospray source.

Protocol

Materials and methods

1. Yeast strains and growth conditions
The wild-type strain BY4742 (*MAT α his3 Δ 1 leu2 Δ 0 lys2 Δ 0 ura3 Δ 0*) was grown in rich YEPD medium (1% yeast extract, 2% peptone, 2% glucose). Cells were cultured at 30°C with rotational shaking at 200 rpm in Erlenmeyer flasks at a "flask volume/medium volume" ratio of 5:1.
2. Storage of yeast cells for lipid extraction
 1. A 50 ml culture of cells was harvested by centrifugation at 3000 x g for 5 minutes.
 2. Washed two times with cold water.
 1. Resuspend pellet in 25 ml ice cold water
 2. Pellet cells in centrifuge at 3000 x g for 5 minutes
 3. Resuspend the pellet and transfer to eppendorf tube
 4. Pellet by centrifugation, 16,000 x g for 2 minutes, remove supernatant and freeze at -80°C until use. (we use a beaker of isopropyl alcohol kept in the -80 freezer, liquid nitrogen can also be used)
3. Reagents
Biotech grade chloroform and methanol were from Sigma-Aldrich. Free fatty acids and triacylglycerols were purchased from Larodan (Malmö, Sweden). Various species of phospholipids - including phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, phosphatidic acid, and cardiolipins - were obtained from Avanti Polar Lipid (Alabaster, AL, USA). High-speed glass centrifuge tubes with Teflon lined caps were from Fisher.

Lipid extraction

This is a modification of the protocol described by Bligh and Dyer⁸. All manipulations with extracted lipids should be done using glass pipettes or syringes; plastics will create a large background signal if they come into contact with chloroform. The exact volumes are not critical as long as the ratios of 1:2:0.8 for chloroform - MeOH - H₂O at step 3 and 2:2:1.8 for chloroform - MeOH - H₂O at step 7 are conserved.

1. Resuspend frozen cells in 1.6 ml of ice-cold distilled H₂O.
2. Transfer 1.6 ml of the cell suspension to high-speed glass centrifuge tubes.
3. Add 6 ml of chloroform and MeOH (1:2) mix and 0.8 ml of glass beads to the cell suspension.
4. Vortex the cell suspension with glass beads two times for 1 min.
5. Add 2 ml of chloroform and mix gently.
6. Incubate for 5 min at room temperature with occasional mixing.
7. Add 2 ml of distilled H₂O and mix gently.
8. Incubate for 5 min at room temperature with occasional mixing.
9. Centrifuge for 5 min at 3000 x g at room temperature.
10. Collect the entire liquid phase into a new high-speed glass centrifuge tube.
11. Add 3.2 ml of chloroform to the cell pellets from step 9.

12. Vortex two times for 1 min.
13. Centrifuge for 5 min at 3000 x g at room temperature.
14. Add the supernatant to the liquid phase from step 10.
15. Centrifuge for 5 min at 3000 x g at room temperature.
16. Discard the upper (aqueous) phase and transfer the lower (organic) phase into a new high-speed glass centrifuge tube.
17. Centrifuge for 5 min at 3000 x g at room temperature.
18. Transfer the entire supernatant into a glass vial and dry under nitrogen.
19. Dissolve the lipid film in 500 µl of chloroform and store at -20°C.

Analysis of lipids by mass spectrometry

A stock mix of lipid standards in chloroform should be prepared beforehand as per Table 1, as well as a stock solution of MeOH - chloroform (1:1) with 0.1% (v/v) ammonium hydroxide.

1. Prior to injection, combine 10 µl of a sample with 10 µl of the standard mix of lipids provided in Table 1. In 200µL of 1:1 chloroform : methanol with 0.1% NH₄OH.
 - The standard to sample ratio can be changed as needed.
2. Resolve lipids using a Micromass Q-TOF 2 mass spectrometer equipped with a nano-electrospray source operating at a flow rate of 1 µl/min.
 - Exact instrument parameters will vary from instrument to instrument. See Table 2 for the settings for a Micromass Q-ToF 2 (Waters, Milford, MA, USA) equipped with a nano-electrospray source. Although we have taken advantage of the high resolution of a Q-TOF type of mass spectrometer, it is not a mandatory requirement.
3. After acquisition the mass spectra are smoothed, background subtracted and centred, and then the peak list exported to Excel. The peaks of each lipid class are then normalized to their internal standard. Depending on the application, it may be necessary to perform further post-processing such as deisotoping and deconvolution.

Table 1. Internal lipid standards, their concentrations in the standard mix, and the MS mode for their analysis.

Lipid class	Standard chain composition	Mass of standard	Concentration (µg/ml)	MS mode
Phosphatidic acid	14:0/14:0	591.40	100	Negative
Phosphatidylethanolamine	14:0/14:0	634.45	200	Negative
Phosphatidylinositol	N/A	N/A	N/A	Negative
Phosphatidylserine	14:0/14:0	622.37	40	Negative
Cardiolipin	4x14:0	619.92	100	Negative
Free fatty acids	19:0	297.28	100	Negative
Phosphatidylcholine	14:0/14:0	650.48	100	Positive
Triacylglycerols	13:0/13:0/13:0	698.63	200	Positive

Table 2. Instrument settings for a Micromass Q-ToF 2 (Waters, Milford, MA, USA) equipped with a nano-electrospray source.

	Flow rate	Cone voltage	Capillary voltage	Collision gas
Positive mode	1µl/min	-28 v	3.0 kv	10
Negative mode	1µl/min	30 v	-3.2 kv	10

Discussion

This method enables a rapid quantitative assessment of the yeast lipidome using readily available, inexpensive materials. The method allows the identification of most of the lipid species found in yeast cells with the exception of diacylglycerols, ergosterols and ergosteryl esters, although these lipids can be identified by replacing ammonium hydroxide with lithium hydroxide. The described method enables lipid identification and quantification at the concentrations as low as µg/ml, with the concentration linearity spreading over 2 to 3 orders of magnitude (depending on lipid species). Although appropriate standards for phosphatidylinositol are not commercially available, the different molecular forms of this phospholipid can be assessed using standards for other phospholipid species.

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