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Lipidomics in research on yeast membrane lipid homeostasis☆

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ABSTRACT

Mass spectrometry is increasingly used in research on membrane lipid homeostasis, both in analyses of the steady state lipidome at the level of molecular lipid species, and in pulse-chase approaches employing stable isotope-labeled lipid precursors addressing the dynamics of lipid metabolism. Here my experience with, and view on mass spectrometry-based lipid analysis is presented, with emphasis on aspects of quantification of membrane lipid composition of the yeast *Saccharomyces cerevisiae*. This article is part of a Special Issue entitled: BBALIP_Lipidomics Opinion Articles edited by Sepp Kohlwein.

1. Introduction

My laboratory investigates the homeostasis of membrane glycerophospholipids (GPL) and is particularly interested in the interplay between GPL class and acyl chain composition. Since lipid metabolism is evolutionarily conserved among eukaryotes [1], we chose to study the processes and regulatory mechanism(s) that govern membrane lipid composition in the reference eukaryote *Saccharomyces cerevisiae*. Besides genetic tractability and the available molecular biology toolbox, yeast offers additional advantages for investigating lipid homeostasis. Yeast is very tolerant with respect to its membrane lipid composition, allowing us to study compensatory mechanisms in yeast mutants that lack one or more enzymes catalyzing bulk lipid synthesis. Moreover, compared to higher eukaryotes the yeast lipidome is relatively simple, facilitating mass spectrometry-based lipid analysis. Yeast has a limited complement of acyl chains with mainly C16 and C18 bearing one or no double bond [2], and it lacks plasmalogens, ether lipids, sphingomyelin and the complex glycosphingolipids found in higher eukaryotes. Arguably, the flexibility of the yeast lipidome could be considered a disadvantage of yeast as model organism for investigating lipid homeostasis, since higher eukaryotes keep membrane lipid class composition in a much tighter range.

Membrane GPL homeostasis is maintained by de novo synthesis, intracellular lipid transport, acyl chain remodeling, and degradation of lipid molecules [3]. Mass spectrometry (LC-MS and ESI-MS/MS) is our method of choice for recording the steady state lipidome of a cell or subcellular organelle at the level of the lipid molecular species. The dynamics of the processes contributing to the steady state lipidome can be conveniently detected in pulse-chase experiments using stable isotope-labeled lipid precursors and subsequent detection by mass spectrometry (ESI-MS/MS). The latter approach known as dynamic lipidomics [4], allows for monitoring the time dependent evolution of lipid molecular species profiles and provides insight into the substrate use (specificity/selectivity) of lipid biosynthetic enzymes [5] and into the occurrence of lipid acyl chain remodeling [6,7]. Here, I will briefly describe our experience with and my view on (dynamic) lipidomics approaches in yeast with emphasis on aspects of the quantification of molecular species of bulk membrane lipids.

2. Experimental procedures

To make the yeast lipidome accessible for lipid extraction, the yeast cell wall has to be disrupted first. Methods commonly used for this purpose include digestion with zymolyase at 30 °C yielding spheroplasts, and mechanic disruption with glass beads (vortex, beadsbeater) at 0–4 °C yielding cell homogenates. Alternatively, lyophilization of yeast cells followed by sonication in the presence of organic solvents has been used to obtain total lipid extracts [8]. For addressing the cellular lipidome, mechanic disruption and lyophilization are the preferred methods to preclude any influence of the zymolyase treatment. On the other hand for determining cellular lipid content (normalized to e.g. dry weight or protein content) lipid extraction after zymolyase treatment is the method of choice because of the reproducibly high lipid recovery. Obviously, pulse-chase experiments in yeast are not compatible with lipid extraction after zymolyase treatment and require mechanic cell wall disruption. Pulse-chase experiments in spheroplasts immediately followed by subcellular fractionation enable tracking of labeled lipids at the level of organellar membranes [9].

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We routinely use the Bligh and Dyer extraction procedure [10] with slight modification to prepare total lipid extracts of yeast cell homogenates, spheroplasts or derived subcellular fractions. The modification entails the acidification of the aqueous phase with HCl (f.c. 0.1 M) to ensure efficient extraction of the acidic GPL. Furthermore, the combined organic phase is washed with 0.1 M KCl to remove the acid prior to evaporating the solvent.

Subsequently, lipid extracts are analyzed by mass spectrometry. In our research we have been mostly interested in how the molecular species profile of phosphatidylcholine (PC) is attained. Shotgun lipidomics has been used to characterize the molecular species profiles of PC and its metabolic precursor phosphatidylethanolamine (PE) on a triple quadrupole mass spectrometer (ESI-MS/MS). PC is readily detected in the positive ion mode by precursor ion scanning for the abundant fragment ion with *m/z* 184, corresponding to the phosphocholine moiety. PE is detected in the positive mode by neutral loss scanning for 141 amu, corresponding to the phosphoethanolamine moiety.

3. Quantification of MS data

Quantification of MS results into mole percentages of molecular species involves correction of isotopic overlap using the available software, and calibration of the instrument's response, since the efficiencies of ionization and fragmentation decrease with increasing *m/z* [11,12]. In addition, relative signal intensities may depend on the lipid concentration of the sample and the degree of unsaturation [12]. Since we have been interested in the proportions of the various molecular species within a lipid class rather than absolute amounts of particular molecular species, we did not use internal standards. Instead, measurements were calibrated using equimolar mixtures of commercially available PC and PE molecular species in the relevant *m/z* range (between C30 and C36) at the relevant concentration [6,7]. Whenever we do not test or correct for the *m/z* dependent instrument response, MS/MS data are quantified and depicted as percentages of total signal intensity.

Internal standards for each lipid class are commonly used to quantify lipid compositions analyzed by LC-MS or shotgun mass spectrometry and to calculate the stoichiometry between lipid classes in the lipidome of interest. Usually a non-natural lipid species is chosen for each lipid class and added to the yeast cell homogenate prior to extraction [13–15]. The inclusion of 2 internal standards, at the lower and upper limits of the *m/z* range of interest, respectively, allows correction for the *m/z* dependence of signal intensities, and provides response control of individual samples [11,16,17]. Interestingly, the stoichiometry between the metabolically related lipid classes PE, PMME, PDME, and PC can be quantitated in a single mass spectrometric analysis, avoiding lipid class-dependent correction factors. PE, PMME, and PDME are converted to PC using deuterated methyliodide followed by multi precursor ion scanning for the phosphocholine headgroup [18].

To detect and track newly synthesized molecular species of PC, deuterium-labeled methionine and choline, substrates of the methylation of PE and the CDP-choline pathway, respectively, have been applied in pulse-chase experiments [6]. Labeling for 10 min is sufficient for precursor ion scanning for the deuterium-labeled phosphocholine moiety. In these experiments, we used non-labeled PC standards for calibration purposes, assuming a similar instrument response. In support of the assumption, the profile of labeled PC species evolved to the steady state (unlabeled) PC species profile during the chase [7]. This finding also argues against the occurrence of isotope effects in the biosynthetic reactions involved. Nowadays, PC standards with various deuterium-labeled head groups are commercially available. Similarly, PE, phosphatidylserine (PS) and phosphatidylinositol (PI) can be pulsed with stable isotope-labeled head group precursors and tracked at the molecular species level [4,17].

4. Cross platform reproducibility and data presentation

In the pre-lipidomics era a number of different labs analyzed the GPL class composition of wild type yeast using several extraction procedures (e.g. Folch [19], Bligh and Dyer [10], Angus and Lester [20]) and a range of chromatography-based separation methods (e.g. TLC). Subsequent quantification of the phospholipid class composition, by phosphorus content or from steady state radiolabeling, has yielded by and large consistent numbers. PC is usually the most abundant membrane lipid followed by PE and PI while PS and cardiolipin (CL) are present as minor bulk lipids. Similarly, yeast acyl chain composition as determined by gas chromatography (GC) has yielded reproducible and consistent results between labs.

Remarkably, determination of the relative proportions of lipid classes in wild type yeast from the first global yeast lipidome analysis [13] revealed PI rather than PC as the predominant membrane lipid. Possible explanations for the discrepancy with the previous data include the method of lipid extraction. Ejsing et al. recovered 80% of PI in the Bligh and Dyer extraction vs. 95% in their 2-step lipid extraction procedure [13]. Another factor potentially contributing to the high PI content measured may originate from overestimation of the abundance of short chain PI molecular species (C26 and C28) in MS/MS studies. Whereas the proportions of the molecular species of PC and PE obtained by ESI-MS on the one hand and tandem MS methods on the other are similar, the C26 and C28 molecular species of PI appear to be enriched in the MS/MS-based analyses [13,21–23] compared to single stage MS [24–26], possibly due to inadequate correction for the dependence of PI signal intensities on *m/z* value in the former.

What is needed to resolve this issue, is the comparative analysis of a (commercially available) standard yeast lipid extract by the various lipidomics platforms and by the conventional chromatography-based methods (TLC, GC). This would reveal the biases, if any, of the various methods and allow for cross platform calibration.

On the other hand, one could argue that deviations in the absolute numbers assigned to membrane lipid composition are of relative importance, given the dependence of yeast lipid composition on culture conditions, growth phase and strain background [27–30]. Importantly, MS-based approaches are extremely powerful in recording *changes* in the yeast lipidome at the molecular species level, e.g. in response to the transition from fermentation to respiration [31].

In this context the presentation of lipidomics data in heat maps (that has spread from transcriptomics and proteomics studies), deserves mentioning. Heat maps depict the changes in a lipidome versus a control or reference lipidome. To be able to appreciate the changes presented in a heat map, the reference lipidome should also be presented. In the absence of the quantitated reference lipidome, a heat plot may be misleading, since a 20% increase in content of an abundant membrane lipid is often more telling than a 200% increase of a minor membrane lipid. It goes without saying that all raw lipidomics data underlying the presented results should be available to the community as supplementary information.

5. Current limitations and future challenges

From the point of view of studying yeast GPL homeostasis, limitations in MS-based lipidomics are few. Powerful methodology is available to record the full lipidome of wild type yeast and to study the effects of perturbations, such as gene deletions or changes in culture conditions. Although technically feasible [32], position-specific assignment of acyl chains is not (yet) mainstream in lipidomics. Instead, acyl chains at the *sn*-1 and *sn*-2 position of the glycerol backbone can be conveniently distinguished by treating lipid extracts with phospholipase A2 [7].

What is still missing is a database collecting and curating yeast lipidomics data. A yeast lipidome database would first of all be a valuable resource of information for researchers, authors and reviewers. It

will likely prevent the erroneous assignment of peaks in spectra to non-yeast lipids (see references [33,34] for recent examples of yeast lipidomes supposedly containing ether lipids). Moreover, with the amount of lipidomics data rapidly expanding, a database will strongly reduce the risk of repeating analyses already published.

Future challenges are found in dynamic lipidomics and modeling of lipid metabolism. Previously, theoretical models of e.g. yeast sphingolipid metabolism and triacylglycerol homeostasis have been designed that were in agreement with experimental data [35,36]. More recently, a predictive model was constructed for determining the remodeling network of PE, based on pulse chase data obtained in mammalian cells pulsed with deuterium-labeled exogenous PS and PE species [37]. By choosing (combinations of) stable isotope-labeled or non-natural lipid precursors it should be possible to track the fluxes of newly synthesized endogenous glycerophospholipids at the level of molecular species in the metabolic pathways under various conditions. This will yield the parameters required for building a mathematical model encompassing the fluxes of lipid molecular species in the lipid biosynthetic pathways, including acyl chain remodeling, that together account for bulk membrane lipid composition. Ultimately, the model should predict how fluxes shift in response to changing conditions or specific perturbations.

Transparency Document

The Transparency document associated with this article can be found, in online version.

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