

High throughput pulse-chase analysis of metabolite turnover in microorganisms followed by LAESI mass spectrometry

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The metabolome of an organism results from a complex biochemical network of thousands of enzymatic reaction and metabolites. Mass spectrometry (MS) in combination with extraction and separation methods has been successfully applied for the detection, identification, and quantitation of these chemical species. However, these methods are slow and provide limited information on the metabolic fluxes essential for the understanding of disease response to treatment and for mechanism of action studies. Stable isotope pulse-chase analysis can be used to study critical pathways in metabolic networks by monitoring the propagation of isotope tracers using MS.¹ Pulse-chase analysis has been used in the clinical laboratory to determine the turnover rates of peptides in diseases such as amyloidosis.² In the pulse phase of these experiments, cells assimilate the labeled molecules, whereas in the chase phase unlabeled molecules are reintroduced into the cells. Molecular turnover rates and half-lives of the affected molecules can be calculated by measuring the kinetics of these processes.

Laser ablation electrospray ionization (LAESI) MS is a high-throughput ambient ionization technique that can detect metabolites, lipids, and peptides directly from biological samples. In LAESI, mid-infrared laser pulses are coupled into the native water content of the biological samples to produce an ablation plume containing mostly neutral particulates. These neutrals are subsequently ionized by charged electrospray droplets and sampled by a mass spectrometer for analysis.³ Recent introduction of ion mobility separation (IMS) with LAESI has enhanced the molecular coverage and sensitivity of this method by separating isobaric species according to

their drift times (DT).⁴ To explore the metabolic response to environmental changes, LAESI-IMS-MS was used to follow the adaptation of lipid production in a well-studied model organism, *Chlamydomonas reinhardtii*, under altered light conditions.⁵

In this contribution, we present an application of pulse-chase analysis by LAESI-IMS-MS to study the turnover kinetics for ¹⁵N-labeled molecules in *C. reinhardtii*. Turnover rates and half-lives of diverse biomolecular species containing nitrogen, such as metabolites, lipids, and peptides can be rapidly determined by this technique. Stable isotope labeling also aids in the identification of the number of nitrogen atoms present in a molecule.

Wild type *C. reinhardtii* was cultured in tris acetate phosphate (TAP) medium containing [¹⁵N]-ammonium chloride for 96 h to allow for label incorporation. The cell culture was maintained at 27 °C and 80 RPM using an orbital shaker incubator. A light source provided 100 μmol·m⁻²·sec⁻¹ illumination and a 12 h / 12 h light-dark cycle. The chase phase started when the ¹⁵N-TAP was removed and an excess of unlabeled TAP medium was added. At selected time points in the chase phase, cell pellets (~10⁶ cells/mL) were produced by centrifugation followed by immediate liquid nitrogen quenching. The frozen pellets were then analyzed by LAESI-IMS-MS.

Nitrogen isotopologs of labeled metabolites, lipids, and peptides can be differentiated based on their *m/z* values. To minimize interferences by other compounds, e.g., structural isomers, ion peaks with identical DT were selected. To eliminate the dependence on instrumental parameters in IMS, the DT values were converted to collision cross sections (CCS). The two CCS values for the isotopologs of diacylglyceryl-N,N,N-trimethylhomoserine DGTS(18:4/16:0), $CCS = 291.5 \pm 1.9 \text{ \AA}^2$, were within the experimental error. DGTS is a betaine lipid containing nitrogen.

At each chase time point a large dataset was collected and represented as a DT vs. *m/z* plot. Isotopologs were identified by finding doublets in the difference heat plot produced by subtracting DT vs. *m/z* plots at different times in the chase phase. By comparing the fully labeled and un-labeled states (see Figure 1), isotopologs of lyso-lipids, chlorophyll *a*, DGTS lipids, and peptides are visualized.

Turnover rates of several metabolites, lyso-lipids, lipids, and peptides were determined by following the chase phase for 72 h. For example, the half-lives of the identified DGTS lipid species were between 40.2 ± 3.9 h for DGTS(16:2/16:0) and 55.5 ± 2.3 h for DGTS(18:4/16:0), whereas the values for DGTS lyso-lipids ranged between 27.1 ± 2.9 h for lyso-DGTS(16:4) and 49.5 ± 4.1 h for lyso-DGTS(18:4). Compared to lipids, peptide ions exhibit significantly lower intensities requiring the IMS for signal enhancement. This provides insight into the turnover of nitrogen in the peptides detected in *C. reinhardtii*. For example, following the isotopologs of the 4+ and 3+ charge states of a 2834.53 ± 0.02 Da peptide revealed a half-life of 10.4 ± 0.7 h.

In this study, high-throughput pulse-chase analysis using LAESI-IMS-MS gave insight into the turnover of metabolites, lipids and peptides in a model microorganism. The utility of this technique can be extended to other microorganisms, as well as to exploring the fluxes in metabolic networks in health and disease.

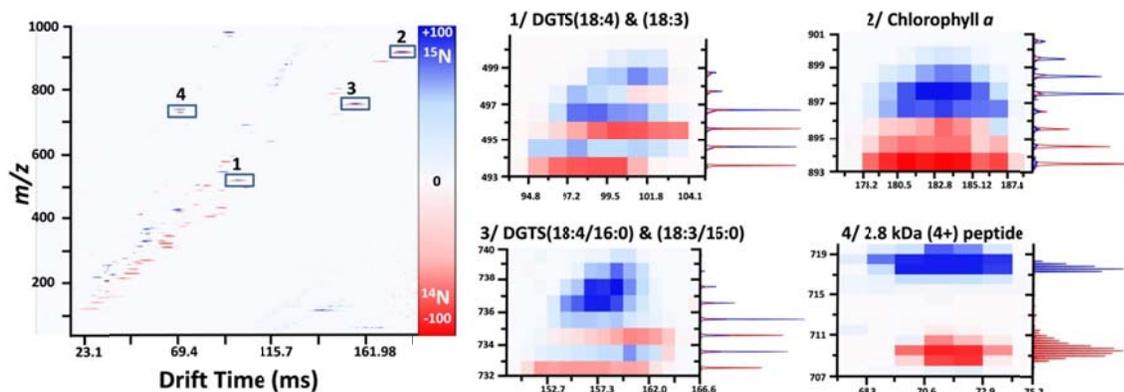


Figure 1. (Left) Difference heat plot, showing ion intensity differences between ^{14}N (red) and ^{15}N (blue) isotopologs. (Right) Zoomed regions show doublets of isotopologs for DGTS lyso-lipids, chlorophyll *a*, DGTS lipids, and a peptide.

References

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