Determination of Metabolite and Lipid Turnover Rates in Live Microalgae by Pulse-Chase Analysis and Laser Ablation Electrospray Ionization Mass Spectrometry

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INTRODUCTION

- The model organism, *Chlamydomonas reinhardtii* has been explored to further understand important biological processes, such as photosynthesis and lipid production, for its relevance as a biofuel source.[1]
- Laser ablation electrospray ionization (LAESI) coupled with ion mobility separation (IMS) for mass spectrometry (MS) has been applied in the profiling of metabolites, lipids, and peptides from cell populations and tissue sections.[2]
- Stable isotope pulse-chase analysis followed by MS allows time course profiling of labeled metabolites, enabling the determination of molecular turnover rates under varied stress conditions.
- In this study, pulse-chase analysis followed by LAESI-IMS-MS (Figure 1) was used to monitor the $^{15}N/^{14}N$ isotope exchange and to determine the turnover rates of nitrogen containing compounds in small cell populations of *C. reinhardtii*.

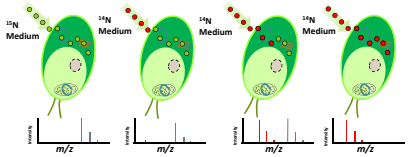


Figure 1. Schematic of pulse-chase analysis. In the pulse phase, *C. reinhardtii* cells are inoculated in an ^{15}N-labeled TAP medium. Within the chase phase, an excess of ^{14}N medium is introduced. Over time, nitrogen containing compounds undergo an ^{15}N to ^{14}N isotope exchange that is analyzed using LAESI-IMS-MS.

METHODS

- Wild type *C. reinhardtii* was cultured in tris-acetate phosphate (TAP) medium at a constant temperature (27 °C) in an orbital shaker (80 RPM) incubator under a 12 h light (100 $\mu mol \cdot m^{-2} sec^{-1}$) - 12 h dark cycle.
- In the pulse phase, cells were cultured in ^{15}N-labeled TAP medium for 96 h. The chase phase was initiated by replacing the ^{15}N-labeled medium with an excess of ^{14}N-TAP medium.
- During the chase phase, sampling was performed at various time points by mid-IR laser ablation (2.94 μm wavelength and 5 ns pulse length) of the cell pellets. The generated plume was ionized by an electrospray, and a time-of-flight MS equipped with traveling-wave IMS analyzed the *m/z* and drift times (DT) of individual ions, respectively.
- Following the time course of exchanging the ^{15}N-labeled metabolites and lipids in the sample by their ^{14}N counterparts provided insight into the turnover rates of these molecules.

RESULTS AND DISCUSSION

PULSE-CHASE ANALYSIS

- Both ^{15}N-labeled and ^{14}N media provided similar growth results for the *C. reinhardtii* cells. The mass spectra of cells cultured in ^{15}N-labeled and ^{14}N media, obtained by LAESI-MS, show similar ion distributions in both environments (Figure 2a).
- The number of nitrogen atoms present in the compound were determined by the shift in *m/z* upon isotope substitution. For example, the incorporation of a single ^{15}N was found in highly abundant nitrogen containing lipids, diacylglyceryl-N,N,N-trimethylhomoserines (DGTS), in *C. reinhardtii* cells and was verified by tandem MS (see Figure 2b).

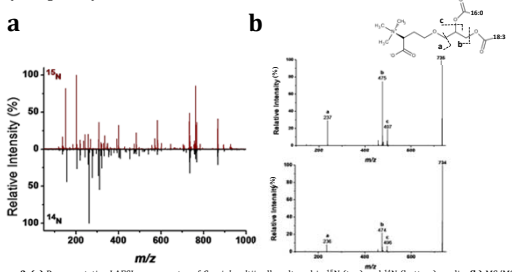


Figure 2. (a) Representative LAESI mass spectra of *C. reinhardtii* cells cultured in ^{15}N (top) and ^{14}N (bottom) media. **(b)** MS/MS spectra of DGTS (18:3/16:0) lipid species cultured in ^{15}N-TAP (top) and ^{14}N-TAP (bottom) media show successful incorporationod a single ^{15}N into the head group of DGTS (18:3/16:0) with a 1 amu shift in the *m/z* value.

- To visualize differences between the labeled and unlabeled compounds, HDMS Compare was employed. Ions with the same DT but with *m/z* differing by a small integer revealed the number of replaceable N atoms in the molecule. For example, ^{14}N and ^{15}N chlorophyll *a* ions exhibited the same DT, but the *m/z* value was shifted by 4 units due to the presence of 4 N atoms in the molecule (Figure 3).

LIPID AND PEPTIDE KINETICS

- Time course profiles were obtained by LAESI-MS analysis for the exchange from ^{15}N to ^{14}N in N-containing compounds. Turnover rates and half-lives were then calculated, e.g., for two DGTS lipids that were detected in *C. reinhardtii* cells (Figure 4).
- Signal enhancement of low abundance peptides was observed by LAESI-IMS-MS. A 2.8 kDa peptide was only detected after IMS separation. The pulse-chase analysis revealed the incorporation of 36 nitrogen atoms into this molecule (Figure 5).

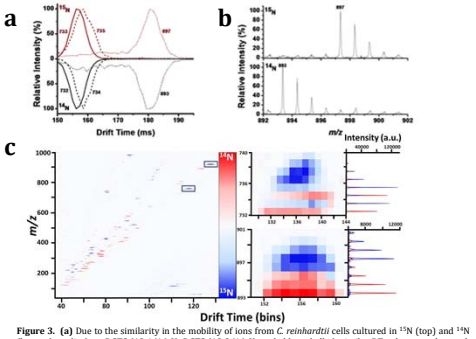


Figure 3. (a) Due to the similarity in the mobility of ions from *C. reinhardtii* cells cultured in ^{15}N (top) and ^{14}N (bottom) media (e.g., DGTS (18:4/16:0), DGTS (18:3/16:0), and chlorophyll *a*), similar DT values are observed. **(b)** Corresponding chlorophyll *a* mass spectra, i.e., (DT=178-183 ms) for *m/z* 897 in ^{15}N-TAP (top) and *m/z* 893 in ^{14}N-TAP (bottom), show no overlap. **(c)** A heat plot, revealing the intensity differences between the ions from cells cultured in ^{15}N (blue) and ^{14}N (red) media, helps to identify nitrogen containing molecules. Zoomed plots for the boxed areas and the corresponding mass spectra are displayed to the right.

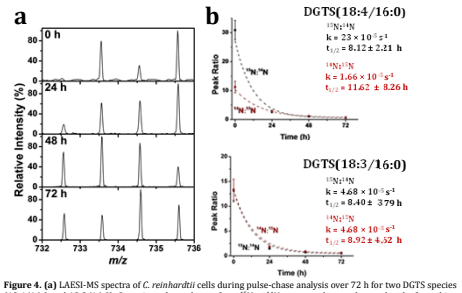
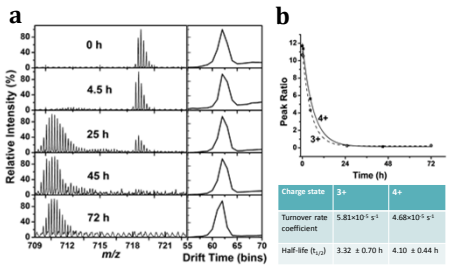


Figure 4. (a) LAESI-MS spectra of *C. reinhardtii* cells during pulse-chase analysis over 72 h for two DGTS species (18:4/16:0 and 18:3/16:0). Over time, the exchange from ^{15}N to ^{14}N compounds was observed and, after taking into account the natural ^{13}C isotope intensities, both species indicated a full conversion within 48 h. **(b)** Kinetics of DGTS (18:4/16:0) (top) and DGTS (18:3/16:0) (bottom) conversion is shown with the corresponding turnover rates and half-lives. The black curves represent the chase phase, whereas the red curves correspond to the pulse phase. In both cases the conversion rates were similar.



Charge state	3+	4+
Turnover rate coefficient	5.81×10^{-5} s^{-1}	4.68×10^{-5} s^{-1}
Half-life ($t_{1/2}$)	3.32 ± 0.70 h	4.10 ± 0.44 h

Figure 5. (a) LAESI-IMS-MS spectra of the 4+ charge state of a 2.8 kDa peptide followed in *C. reinhardtii* during pulse-chase analysis over a 72 h time period. Corresponding DT window for the selected peptide ion (4+) for each time point is shown at the right. **(b)** Kinetics of the peptide turnover for the two charge states with corresponding turnover rates and half-lives.

CONCLUSIONS

- Pulse-chase method followed by LAESI-IMS-MS can add insight into metabolic network dynamics and molecular turnover rates in live biological systems.
- For the detection of ^{15}N to ^{14}N kinetics, IMS was a valuable tool by reducing isobaric interferences and aiding in the detection of low abunadance species.

ACKNOWLEDGMENTS

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