Metabolite and Lipid Turnover Rates Determined by Pulse-Chase Analysis and Laser Ablation Electrospray Ionization Mass Spectrometry

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INTRODUCTION

- *C. reinhardtii* has been used as a model organism to study cell cycle, photosynthesis, genetic modifications[1], and its relevance for biofuels.
- Stable isotope pulse-chase analysis followed by mass spectrometry allows for time course profiling and determination of molecular turn-over rates.
- Laser ablation electrospray ionization (LAESI) coupled with ion mobility separation (IMS) mass spectrometry (MS) (see Figure 1) has shown success in high-throughput detection of metabolites, lipids, and peptides from cell population and tissue sections.[2]
- In this study, pulse-chase analysis followed by LAESI-IMS-MS was used for the monitoring of isotope exchange and to determine turnover rates in nitrogen containing compounds in *C. reinhardtii* cell populations.

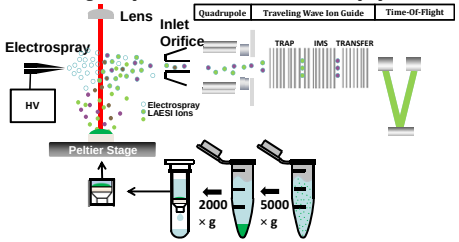


Figure 1. Schematic representation of analysis of *C. reinhardtii* cells by LAESI-IMS-MS.

METHODS

- Wild type *C. reinhardtii* were inoculated 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 μmol·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 the removal of the ^{15}N labeled medium and exchange with the ^{14}N-TAP.
- Sampling was performed at various time points during the chase phase by directly coupling a 2.94 μm wavelength 5 ns laser pulse into the native water found in the cell pellets. The subsequent ablation 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.

RESULTS

ISOTOPE INCORPORATION

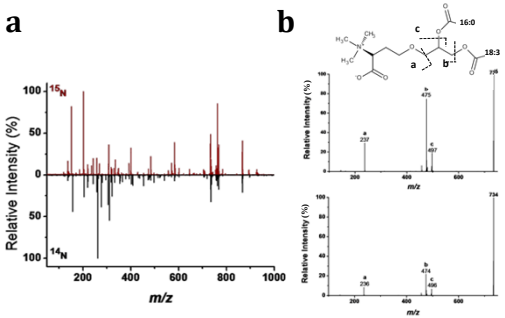


Figure 2. (a) Representative LAESI mass spectra of *C. reinhardtii* cells cultured in ^{15}N-TAP (top) and ^{14}N-TAP (bottom) media. **(b)** MS/MS spectra of DGTS (18:3/16:0) lipid species cultured in ^{15}N-TAP (top) and ^{14}N-TAP (bottom) media show successful incorporation of ^{15}N into the head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

LIPID TURNOVER

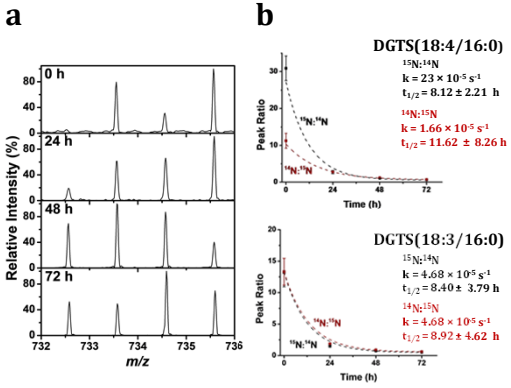


Figure 4. (a) LAESI-MS spectra of *C. reinhardtii* cells during pulse-chase analysis over 72 h for two DGTS species (18:4/16:0 and 18:3/16:0). Over time the exchange from ^{15}N to ^{14}N compounds was observed and, after taking into account the natural ^{13}C isotope intensities, both species had a 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.

PULSE-CHASE ANALYSIS WITH IMS

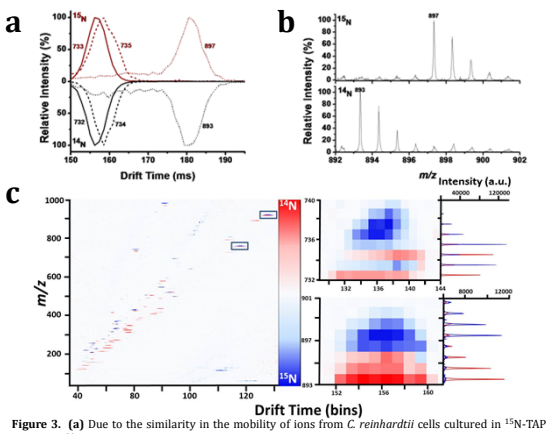
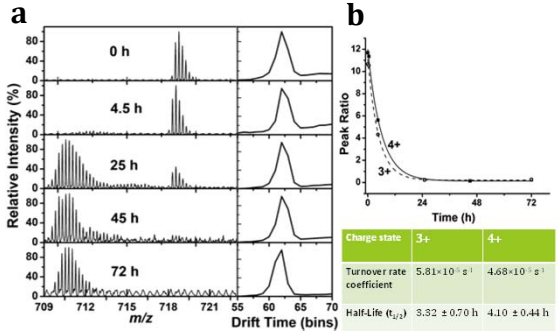


Figure 3. (a) Due to the similarity in the mobility of ions from *C. reinhardtii* cells cultured in ^{15}N-TAP (top) and ^{14}N-TAP (bottom) (e.g., DGTS (18:4/16:0), DGTS (18:3/16:0), and chlorophyll *a*), similar DT values are observed. **(b)** Corresponding chlorophyll *a* mass spectra, i.e., (DT=178-183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

PEPTIDE TURNOVER



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 two charge states with corresponding turnover rates and half-lives.

DISCUSSIONS

- Both ^{15}N-labeled and ^{14}N-TAP media provided similar growth conditions for *C. Reinhardtii* cells and showed the incorporation of ^{15}N in the pulse phase (Figure 2).
- HDMS Compare was employed to visualize differences between the unlabeled and labeled compounds. 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 were exhibiting the same DT, but the *m/z* value was shifted by 4 unites due to the presence of 4 N atoms in the molecule (Figure 3).
- Turnover rates and half-lives of two DGTS lipids were determined using the LAESI-MS data alone (Figure 4).
- Signal enhancement of low concentration peptides with LAESI-IMS-MS was observed. The 2.8 kDa peptide was only detected after IMS separation. The pulse-chase analysis revealed the incorporation of 36 nitrogen atoms into the molecule (Figure 5).

CONCLUSIONS

- The kinetics of lipid and peptide turnover were determined by using pulse-chase experiments monitored by LAESI-IMS-MS in *C. reinhardtii* cell pellets over a 72 h chase phase.
- IMS was found to be a valuable tool in the detection ions for the ^{15}N to ^{14}N kinetics by reducing isobaric interferences and aiding in the detection of low abunadance species.
- LAESI-IMS-MS in combination with pulse-chase analysis can add insight into metabolic network dynamics and molecular turnover rates in live biological systems.

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