High Throughput Pulse-chase Analysis of Metabolite Turnover in Microorganisms by LAESI Mass Spectrometry

Sylwia A. Stopka,[a] Tarek Mansour,[a] Bindesh Shrestha,[a] Éric Maréchal,[b] Denis Falconet,[b] and Akos Vertes[a]

[a]Department of Chemistry, W. M. Keck Institute for Proteomics Technology and Applications, The George Washington University, Washington, DC 20052
[b]Laboratoire de Physiologie Cellulaire Végétale, UMR 5168, CEA-CNRS-INRA-Université Joseph Fourier Grenoble 1, Grenoble, France

INTRODUCTION

- Stable isotope pulse-chase analysis followed by mass spectrometry (MS) can provide insight into cellular metabolism kinetics from complex biological systems.
- The ambient ionization source, laser ablation electrospray ionization (LAESI) in combination with gas-phase ion mobility separation (IMS)-MS has demonstrated high-throughput detection of metabolites, lipids, and peptides from microalgae cell populations.[1]
- The model organism, *Chlamydomonas reinhardtii* has been extensively studied for its well known lipid metabolism, chlorophyll cycle, and use in biofuel alternatives.
- In this study, pulse-chase analysis in combination with LAESI-IMS-MS was used for the simultaneous and rapid determination of molecular turnover rates and half-lives in live microalgae.

METHODS

- Wild type *C. reinhardtii* were inoculated in tris-acetate phosphate (TAP) medium at 27 °C and 80 RPM with 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 to allow isotope assimilation. The chase phase was initiated when the medium was reverted to unlabeled TAP.
- During the chase phase, algae were analyzed using LAESI-IMS-MS at several time points. Briefly, mid-IR laser pulses were focused onto the cell pellet to produce an ablation plume that was subsequently ionized by an electrospray. A traveling-wave IMS equipped TOF-MS detected the isotopolog *m/z* values and drift times (DT). The overall workflow is shown in Figure 1.

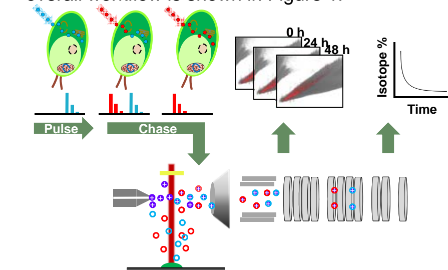


Figure 1. Schematic representation of the workflow of pulse-chase analysis with LAESI-IMS-MS for live *C. reinhardtii* cells.

RESULTS

ISOTOPE ASSIMILATION

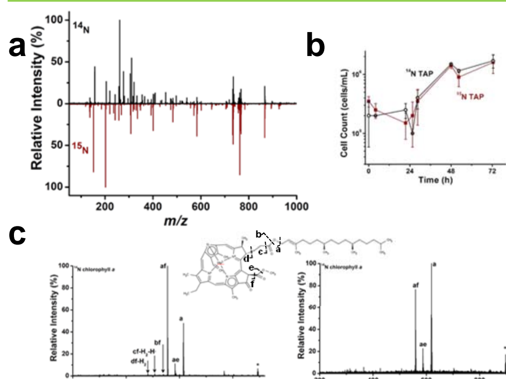


Figure 2. (a) Representative LAESI mass spectra of *C. reinhardtii* cells cultured in (top) unlabeled-TAP and (bottom) ^{15}N-TAP media. **(b)** The growth rates of algae were not affected by the ^{15}N-labeled isotope. **(c)** Tandem MS of chlorophyll *a* isotopologs from *C. reinhardtii* in (left) unlabeled and (right) ^{15}N-labeled media. Inset shows the structure and fragmentation of chlorophyll *a*.

ISOTOPOLOG IMS ENHANCEMENT

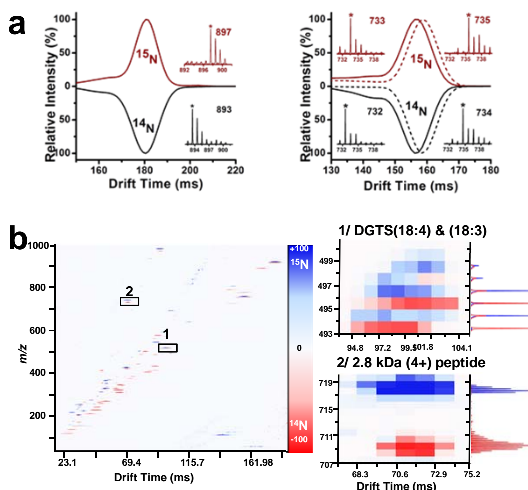


Figure 3. (a) Isotopolog DT distribution plots of (left) chlorophyll *a* and (right) a 2.8 kDa peptide from *C. reinhardtii* in unlabeled and ^{15}N-labeled media. **(b)** A difference heat plot, revealing the intensity differences between the ions from (blue) ^{15}N-TAP and (red) unlabeled TAP media. Isotopolog doublets (right) in the zoomed plots were observed with similar DT ranges and shifted *m/z* values determined by the number of nitrogen atoms.

METABOLITE TURNOVER

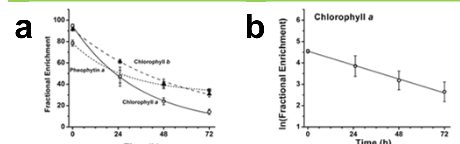
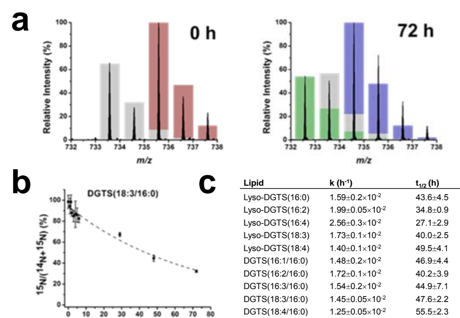


Figure 4. (a) Fractional enrichment of the decay for pheophytin *a* (□), chlorophyll *b* (▲), and chlorophyll *a* (○) over time. **(b)** Chlorophyll *a* follows first order kinetics during the 72 h chase phase. Similar trends where observed for the other two.

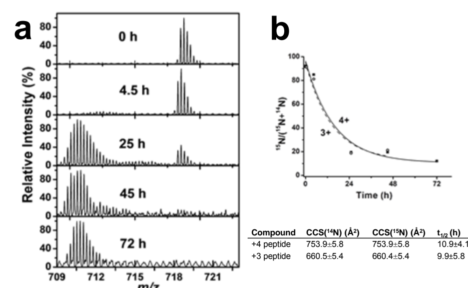
LIPID KINETICS



Lipid	k (h^{-1})	$t_{1/2}$ (h)
Lyso-DGTS(16:0)	1.59±0.2×10^{-2}	43.6±4.5
Lyso-DGTS(16:2)	1.99±0.05×10^{-2}	34.8±0.9
Lyso-DGTS(16:4)	2.56±0.3×10^{-2}	27.1±2.9
Lyso-DGTS(18:3)	1.73±0.1×10^{-2}	40.0±2.5
Lyso-DGTS(18:4)	1.40±0.1×10^{-2}	49.5±4.1
DGTS(16:1/16:0)	1.48±0.2×10^{-2}	46.9±4.4
DGTS(16:2/16:0)	1.72±0.1×10^{-2}	40.2±3.9
DGTS(16:3/16:0)	1.54±0.2×10^{-2}	44.9±7.1
DGTS(18:3/16:0)	1.45±0.05×10^{-2}	47.6±2.2
DGTS(18:4/16:0)	1.25±0.05×10^{-2}	55.5±2.3

Figure 5. (a) Isotope distribution patterns during a 72 h chase phase for diacylglyceryl N,N,N-trimethylhomoserines (DGTS) DGTS(18:4/16:0) and DGTS(18:3/16:0). Experimental spectra are shown as black traces and green/blue colors are designated as the unlabeled isotopologs of DGTS(18:4/16:0) and DGTS(18:3/16:0), respectively. The grey/red colors represent the ^{15}N-labled isotopologs. **(b)** Fractional enrichment of DGTS(18:3/16:0) ion over a 72 h chase period. **(c)** Turnover rates and half-lives of lipids detected from *C. reinhardtii.*

PEPTIDE TURNOVER



Compound	CCS(^{14}N) (Å^2)	CCS(^{15}N) (Å^2)	$t_{1/2}$ (h)
+4 peptide	753.9±5.8	753.9±5.8	10.9±4.1
+3 peptide	660.5±5.4	660.4±5.4	9.9±5.8

Figure 6. (a) LAESI-IMS-MS spectra during a 72 h pulse-chase experiment within the DT range of 60-62 ms, which provided signal enhancement for a 2.8 kDa (4+ charge state) peptide from a *C. reinhardtii* cell pellet. **(b)** Fractional enrichment for the 2.8 kDa peptide in both the +4 and +3 charge state and collision cross sections and half-lives values.

DISCUSSIONS

- Nitrogen isotope assimilation had no effect on cellular growth, and showed spectral features similar to the unlabeled condition (Figure 2).
- Collision cross section (CCS) measurements based on ion mobility separation aided in confirming isotopologs. For example, isotopologs of chlorophyll *a* ions exhibited the same CCS, but the *m/z* value was shifted by 4 unites due to the presence of four nitrogen atoms in the molecule. To detect isotopologs, a difference heat plot was constructed, showing doublets. (Figure 3)
- The decomposition kinetics of porphyrins were determined. They followed first-order decay during the 72 h chase phase. The half-life of, chlorophyll *a* was 24.1±2.2 h, chlorophyll *b* was 44.7±1.6 h, and pheophytin *a* was 18.9±2.7 h. (Figure 4)
- The turnover rates and half-lives of lyso-DGTS and DGTS lipids provided a tool for investigating lipid metabolism. (Figure 5)
- Signal enhancement of low concentration isotopolog peptides was observed by introducing IMS. A 2.8 kDa peptide contained 36 nitrogen atoms and had a half-life of 10.9±4.1 h. (Figure 6).

CONCLUSIONS

- Pulse-chase analysis followed by *LAESI-IMS-MS* demonstrated the feasibility of simultaneous rapid determination of turnover rates and half-lives for metabolites, lipids, and a peptide in a single experiment.
- The enhancements due to IMS allowed for the, separation of isotopologs, detection of low abunadance species, and strenghtening identifications using CCS values.
- This work benefits systems biology and bioengineering, and provides further insight into lipid metabolism in complex biological systems.

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[1] S. A. Stopka, B. Shrestha, E. Marechal, D. Falconet, A. Vertes, *Analyst* **2014**, 139, 5945-5953.