Metabolomic and Lipidomic Analysis of Live Microalgae by Laser Ablation Electrospray Ionization Mass Spectrometry with Ion Mobility Separation

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Overview

Laser ablation electrospray ionization (LAESI) mass spectrometry (MS) equipped with ion mobility separation (IMS) was used to detect metabolomic and lipidomic changes in live populations of microalgae (*Chlamydomonas reinhardtii*) growing in autotrophic and heterotrophic conditions.

Introduction

- *C. reinhardtii* has been studied as a model organism for photosynthesis, genetic modification, cell cycle, and motility.[1] Due to the excessive production of hydrogen and lipids, it has been considered as an alternative biofuel source.
- LAESI-MS has been successful for the detection of small metabolites, lipids, proteins from small cyanobacterial populations,[2] single plant cells , etc., in ambient conditions.
- In this study, LAESI-IMS-MS was applied for ambient analysis of molecular changes in the low and intermediate mass-to-charge (*m/z*) regions of live *C. reinhardtii* populations.

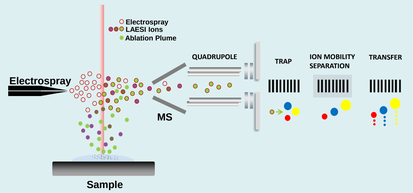

Figure 1. Schematic representation of LAESI-IMS-MS with dual collision induced dissociation (CID).

Chlamydomonas reinhardtii

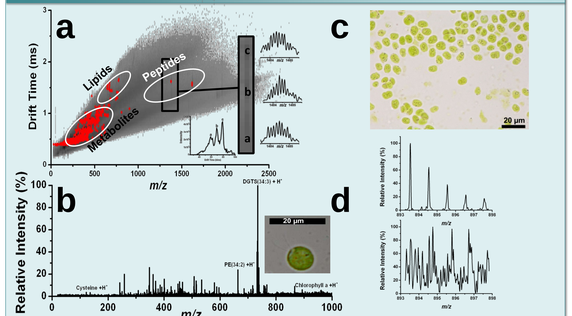

Figure 2. (a) Drift time (DT) vs. *m/z* map of *C. reinhardtii* cells with highlighted regions corresponding to metabolites, lipids, and peptides. The insets show three distinct peaks in the DT distribution for a multiply charged ion at *m/z* 1484, indicating three structural conformers. **(b)** Mass spectrum of *C. reinhardtii* cells averaged over the entire DT range with **(c)** a representative microscope image. **(d)** LAESI mass spectrum at the DT = 1.40 ms shows chlorophyll *a* ion (top), which is indistinguishable without IMS (bottom).

Methods

- Wild type *C. reinhardtii* (cc125 m+) was inoculated in tris-acetate phosphate medium and cultured at 23°C on a shaker under 15 μmol photons $m^{-2}s^{-1}$.
- LAESI-IMS-MS sampling was performed by coupling mid-IR laser pulses (λ= 2.94 μm, 5 ns pulse width, 20 Hz repetition rate) directly into a microalgae pellet suspended in deionized water.
- The ablation plume was intercepted and ionized by electrospray droplets. The resulting ions were sampled by a high performance quadrupole time-of-flight mass spectrometer with traveling-wave IMS. Orthogonal projections to latent structures discriminant analysis was performed for comparative studies.
- Time-aligned parallel (TAP) fragmentation method was utilized for structural elucidation by the production of 1st and 2nd generation fragments. See Figure 1.

Structural Elucidation

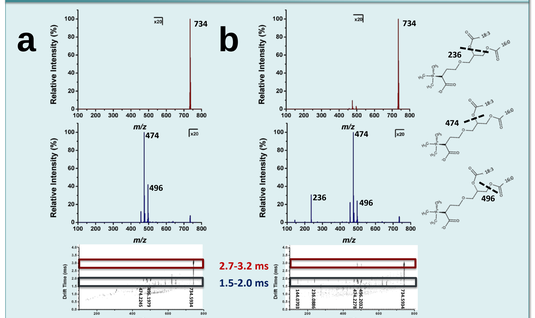

Figure 3. (a) For MS/MS of the *m/z* 734 ions, LAESI mass spectra of the first generation fragment ions at DT 2.7 to 3.2 ms (top) and DT 1.5 to 2.0 ms (middle) are shown with the DT vs. *m/z* map (bottom). **(b)** Similarly, mass spectra of the second generation ions with DT aligned fragments give MS/MS/MS spectra for the *m/z* 734 ion confirming its identity as DGTS(16:0/18:3).

Lipidomic Changes

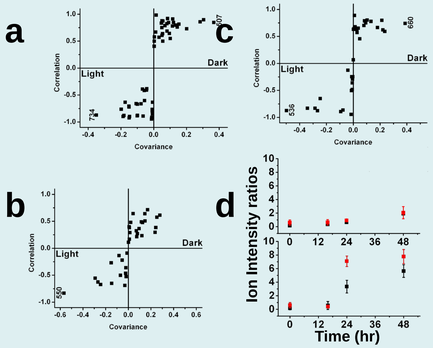

Figure 4. S-plots showing changes in the lipid region of *C. reinhardtii* spectra in light (negative-axis) and dark (positive-axis) environment at **(a)**16 hr, **(b)** 24 hr, and **(c)** 48 hr. **(d)** Intensity ratio of lipid ions for *m/z* 660-734 (black) and 507-660 (red) in light (top) and dark (bottom) environment. The intensity ratios indicate uniform lipid intensities under light conditions (top), but up to an 8-fold increase in the dark (bottom).

Autotrophic and Heterotrophic Conditions

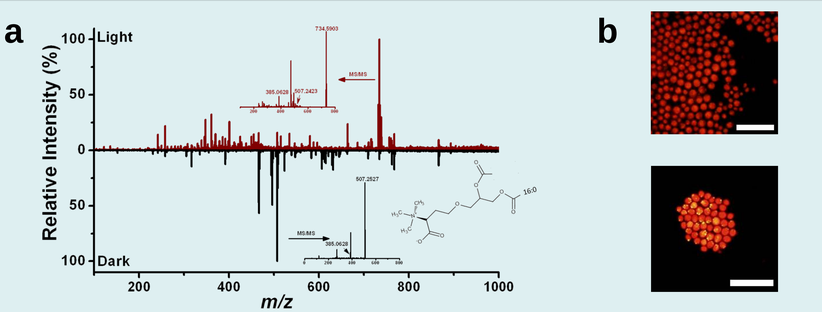

Figure 5. (a) LAESI mass spectra of *C. reinhardtii* cultured in light (top) and dark (bottom) conditions for 16 hours. Top and bottom insets are tandem MS of *m/z* 734 (DGTS) and 507, respectively. Ion at *m/z* 507 is a fragment of *m/z* 734. Increase of DGTS fragment ion at *m/z* 507 in dark conditions and decrease of *m/z* 734 hints possible lipid degradation. **(b)** Fluorescence images of *C. reinhardtii* stained with Nile red in 16 hour light (top) and dark (bottom). The red fluorescence indicates chlorophyll auto-fluorescence while yellow fluorescence found in *C. reinhardtii* grown in dark corresponds to neutral lipids. Scale bars are 50 μm.

Conclusions

- We showed direct analysis of metabolite and lipid species in live *C. reinhardtii* cell populations with LAESI-IMS-MS.
- Time-aligned parallel fragmentation allowed for high-throughput structural identification of the head-group and acyl chain length of lipid ions.
- IMS improves signal-to-noise ratio due to the reduction of isobaric interferences, aiding in the detection of low abundance metabolites, lipids, and peptides.
- LAESI-IMS-MS shed new light on metabolic and lipid changes as a function of light conditions.

References

[1] Merchant, S. S., *et.al.*, "The Chlamydomonas Genome Reveals the Evolution of Key Animal and Plant Functions", *Science*, **2007**, 318, 245-250.

[2] Shrestha, B.; Parsiegla, G.; Carrière, F.; Vertes, A., "Direct Analysis of Phycobilisomal Antenna Proteins and Metabolites in Small Cyanobacterial Populations by Laser Ablation Electrospray Ionization Mass Spectrometry", *Analytical Chemistry*, **2011**, 84, 34-38.

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