Analytica Chimica Acta 902 (2016) 1–7

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Turnover rates in microorganisms by laser ablation electrospray ionization mass spectrometry and pulse-chase analysis

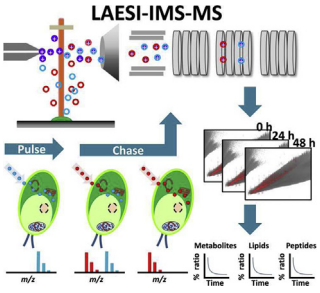
Sylwia A. Stopka[a], Tarek R. Mansour[a], Bindesh Shrestha[a], Éric Maréchal[b], Denis Falconet[b], Akos Vertes[a,*]

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

HIGHLIGHTS

- High-throughput pulse-chase analysis using direct sampling of biological cells.
- Ion mobility separation for the elucidation of isotopologs.
- Identification of isotopologs in difference heat plots of DT vs. *m*/*z*.
- Simultaneous determination of turnover rates for lipids and peptides in microalgae.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 2 June 2015
Received in revised form 20 August 2015
Accepted 23 August 2015
Available online 1 September 2015

Keywords:
Turnover rate
Cell
Mass spectrometry
Pulse-chase analysis
Laser ablation electrospray ionization

ABSTRACT

Biochemical processes rely on elaborate networks containing thousands of compounds participating in thousands of reaction. Rapid turnover of diverse metabolites and lipids in an organism is an essential part of homeostasis. It affects energy production and storage, two important processes utilized in bioengineering. Conventional approaches to simultaneously quantify a large number of turnover rates in biological systems are currently not feasible. Here we show that pulse-chase analysis followed by laser ablation electrospray ionization mass spectrometry (LAESI-MS) enable the simultaneous and rapid determination of metabolic turnover rates. The incorporation of ion mobility separation (IMS) allowed an additional dimension of analysis, i.e., the detection and identification of isotopologs based on their collision cross sections. We demonstrated these capabilities by determining metabolite, lipid, and peptide turnover in the photosynthetic green algae, *Chlamydomonas reinhardtii*, in the presence of ^{15}N-labeled ammonium chloride as the main nitrogen source. Following the reversal of isotope patterns in the chase phase by LAESI-IMS-MS revealed the turnover rates and half-lives for biochemical species with a wide range of natural concentrations, e.g., chlorophyll metabolites, lipids, and peptides. For example, the half-lives of lyso-DGTS(16:0) and DGTS(18:3/16:0), $t_{1/2}$ = 43.6 ± 4.5 h and 47.6 ± 2.2 h, respectively, provided insight into lipid synthesis and degradation in this organism. Within the same experiment, half-lives for chlorophyll *a*, $t_{1/2}$ = 24.1 ± 2.2 h, and a 2.8 kDa peptide, $t_{1/2}$ = 10.4 ± 3.6 h, were also determined.

http://dx.doi.org/10.1016/j.aca.2015.08.047
0003-2670/

1. Introduction

Metabolic network reconstructions with thousands of metabolites and reactions exhibit remarkable complexity. For example, the iRC1080 reconstruction of the *Chlamydomonas reinhardtii* pathways accounts for 1068 metabolites and 2190 reactions in 10 cellular compartments [1]. Understanding the homeostasis of organisms, based on a robust regulation of these networks, requires detailed information on the turnover of many biochemical species. The main factors defining turnover are the existing pools of molecules and the net sum of the incoming and outgoing fluxes.

Over the years stable isotope labeling has been incorporated in metabolic studies to improve metabolite identification, perform quantification, and obtain reaction kinetics information for biochemical pathways [2]. Dynamic changes in labeled chemical species in metabolic networks are most often followed by mass spectrometry. Monitoring the propagation of isotope labeling through the metabolic network in combination with perturbations, e.g., genetic knockouts or a drug treatment, on well-known pathways, such as the central carbon metabolism (tricarboxylic acid cycle, glycolysis, and pentose phosphate pathway), provide information on metabolic fluxes in health and disease [3]. New metabolic networks and connections can be mapped by monitoring the incorporation of the labels [4]. Other applications of pathway analysis and genetic manipulation include metabolic engineering to promote the production of high value chemicals by microorganisms [5,6].

Stable isotope labeling of amino acids in cell cultures (SILAC), where ^{13}C-labeled essential amino acids are incorporated in the medium, is predominantly used for labeling of metabolites in mammalian cells [7]. A similar approach can be applied to label plant cells, where salts and/or sugars containing ^{13}C or ^{15}N isotopes can be incorporated in the media [8–10]. This technique has been applied in model systems including *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, *Arabidopsis thaliana*, and *C. reinhardtii* [11–13].

Pulse-chase analysis consists of two phases [14]. In the pulse phase, cells are cultured in a labeled medium, whereas in the chase phase, the medium is reverted to the unlabeled form. During the latter phase, the time dependence of isotopolog composition is determined by mass spectrometry (MS). Due to its high sensitivity and wide dynamic range, MS in combination with chromatographic separation methods is increasingly used for turnover measurements [15]. Powerful methods, e.g., GC–MS with stable isotope labeling, exist for the simultaneous determination of multiple metabolite fluxes in microbial systems [16–18]. However, due to the long retention times, time consuming extraction, hydrolysis and derivatization protocols in conventional separations, and the large quantity of species required to be labeled, result in low throughput.

The ambient ionization technique, laser ablation electrospray ionization (LAESI) MS, has been used for the rapid detection and identification of diverse metabolites, lipids, and peptides [19]. Recently coupling ion mobility separation (IMS) to this platform allowed for the high-throughput elucidation of lipids and metabolites in microalgae [20,21]. The analysis time is reduced from the typical ~20 min needed for HPLC to ~200 ms required for an IMS separation.

To demonstrate the utility of this method, *C. reinhardtii* was selected because this photosynthetic organism has a well-studied lipid metabolism [22,23] and has the ability to produce substantial amounts of lipids, which in turn can be processed into biofuels [14]. Using ^{13}C-based methods, e.g., SILAC, would result in reduced levels of labeling (~80%) because *C. reinhardtii* is autotrophic [24–27]. To achieve higher isotope incorporation for molecules containing nitrogen, ^{15}N-ammonium chloride is used in the culture [28]. In this contribution, the first combination of pulse-chase analysis with the LAESI-IMS-MS technique allows for high throughput determination of turnover rates with extensive metabolite and lipid coverage.

2. Experimental

2.1. Chemicals

The 99% enriched [^{15}N]-ammonium chloride was purchased from Cambridge Isotope (Andover, MA). Original Hutner's Trace element stock was obtained from the Chlamydomonas Resource Center at the University of Minnesota. Chlorophyll *a* standard was purchased from Sigma–Aldrich (St. Louis, MO). The 50% methanol electrospray solution contained 0.1% acetic acid that was purchased as glacial acetic acid (≥99.0%) from Fluka (St. Louis, MO). Certified HPLC grade methanol and water were obtained from Alfa Aesar (Ward Hill, MA).

2.2. Growth media

Two types of tris acetate phosphate (TAP) media [29] were made in house, unlabeled containing natural nitrogen isotope distribution and ^{15}N-labled produced by using 99% enriched ^{15}N-labeled ammonium chloride. Both media contained the following components: 2.42 g of tris base, 25.0 mL of Beijerinck salts, 1.0 mL phosphate solution, 1.0 mL Hutner trace element solution, and 1.0 mL glacial acetic acid. The final concentrations were 7 mM NH_4Cl, 830 μM $MgSO_4$, and 450 μM $CaCl_2$ for the Beijerinck salts and 1.65 mM K_2HPO_4 and 1.05 mM KH_2PO_4 for the phosphate solution. The pH values of the media were adjusted to 7.0, and the TAP solutions were diluted to 1000 mL by water. The resulting solutions were autoclaved (Hiclave HVA-85, Hirayama, Concord, CA) at 121 °C for 20 min and stored at 4 °C in a dark environment.

2.3. Cell culture

Wild type *C. reinhardtii* (CC125) stock was purchased from the Chlamydomonas Resource Center at the University of Minnesota. The cells were grown in either ^{15}N-labeled or unlabeled TAP medium at 27 °C while rotated at 80 RPM in an orbital shaker incubator (MaxQ400, Thermo Scientific, Waltham, MA). Two LED light bulbs (daylight white, 6 W and 10 W), producing ~100 $\mu mol \cdot m^{-2} sec^{-1}$ intensity radiation, were installed above the culture. A digital timer provided automated 12 h light/12 h dark cycles. Illumination was monitored using a photosynthetic active radiation (PAR) meter (Sun System, Sunlight Supply Inc., Vancouver, WA). In order to determine growth rates, cells were counted by a hemocytometer (Bright-Line, Horsham, PA).

2.4. Pulse-chase analysis

The experiment started with the pulse phase, in which cells were grown for 96 h in ^{15}N-labeled TAP medium, allowing nitrogen assimilation to label the cells. In the pulse phase high fractional enrichment, $^{15}N/(^{15}N+^{14}N)$, was observed for both chlorophyll *a* (94.6%) and chlorophyll *b* (91.5%), whereas pheophytin *a* was observed at a lower percent (78.4%). Cells were then pelleted by centrifugation at 2000× *g* for 1 min, washed and re-suspended in unlabeled medium, marking the beginning of the chase phase. Sampling at several time points in the chase phase was performed by collecting ~10^6 cells and adjusting the volumes to 1.0 mL. These

* Corresponding author.
E-mail address: vertes@gwu.edu (A. Vertes).