Reactive mass spectrometry determination of C=C bonds in unsaturated lipids in single cells from laboratory experiments performed in 2018 and 2019

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Project
» Collaborative Research: Creatine Cycling in Marine Bacterial and Phytoplankton Assemblages (Creatine Cycling)

Contributors	Affiliation	Role
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Abstract
Reactive mass spectrometry determination of C=C bonds in unsaturated lipids in single cells from laboratory experiments performed in 2018 and 2019. These data were used to create Table 1 in the results publication Zhu et al. (2020).

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Coverage

Temporal Extent: 2018-12 - 2019-06

Methods & Sampling

The following are excerpts from Zhu et al., 2020. Please refer to this publication for more details.

Methodology

Fabrication of the Micropipette Needle. The micropipette needle (tip size ≈ 15 um) was pulled from a glass capillary tube (size: 0.8 × 90 mm2, Kimble Chase Life Science and Research Products, Rockwood, TN) using a pipet puller (KOPF, Tujunga, CA). UV epoxy (Prime-Dent, Chicago, IL) was used to connect the micropipette needle to a fused silica capillary (OD: 150 µm, ID: 75 µm, Polymicro Technologies, Phoenix, AZ). A syringe was connected to the fused silica capillary via a conductive union (IDEX Health & Science LLC, Oak Harbor, WA).

Sampling device

The Micropipette Needle was produced for sampling. The micropipette needle (tip size ≈ 15 um) was pulled from a glass capillary tube (size: 0.8 × 90 mm2, Kimble Chase Life Science and Research Products, Rockwood, TN) using a pipet puller (KOPF, Tujunga, CA). UV epoxy (Prime-Dent, Chicago, IL) was used to connect the micropipette needle to a fused silica capillary (OD: 150 µm, ID: 75 µm, Polymicro Technologies, Phoenix, AZ). A syringe was connected to the fused silica capillary via a conductive union (IDEX Health & Science LLC, Oak Harbor, WA). Using an Eppendorf cell manipulation system and a syringe pump, a target cell was sucked into the glass micropipette (flow rate 10 µL/min) containing prefilled acetone or benzophenone solution. Additional solution was drawn into the micropipette needle to ensure cell lysis. The syringe pump was turned on to deliver (flow rate 0.2 µL/min) the single cell lysate toward the nano-ESI emitter. Both the regular (no UV irradiation) and the reactive (after UV irradiation) single cell MS experiments can be conducted for the same single cell. Specifically, after accomplishing data acquisition of the regular SCMS experiment, the ionization energy was turned off and the syringe pump was paused. The UV lamp (BHK, Ontario, CA) was then turned on to generate UV radiation and initiate PB reactions between the reagents and unsaturated cellular lipids. After 15 min of reaction, the UV light was turned off, and then

the reactive single cell MS experiment was started by turning on the ionization voltage and resuming the syringe pump. Products from the PB reactions were analyzed using both MS scan (to obtain accurate m/z values of all ions) and tandem MS (MS/MS) analysis (to acquire fragments of selected ions).

Instrument

Thermo LTQ Orbitrap XL mass spectrometer (Thermo Scientific, Waltham, MA, United States). Mass analyze parameters were as follows: mass resolution 60,000, 1 microscan, 100 ms max injection time, and automatic gain control on. A DC ionization voltage (+4 kV in the positive ion mode or −4 kV in the negative ion mode) was applied on a conductive union and transmitted through the solvent to induce ionization of cell lysis at the tip of the micropipette for MS analysis.

Location of experiments: University of Oklahoma, Norman, OK 73019

Cell line: HCT-116 (human colon cancer cell line)
Reactions: Paternò-Büchi (PB) between unsaturated lipids (contain carbon-carbon double bonds) and acetone or benzophenone

Data Processing Description

processed using Genna 2 oneline software, unpublished.

BCO-DMO data manager processing notes:
* Loaded Sheet 1 "mz list using Script A" from file "Double bond single cell_BCODMO.xlsx" into the BCO-DMO data system.
* Unpivoted data. Transformed from many mass to charge ratio columns with multiple header rows containing information for Cell number, ion mode, before or after PB reaction into a data table with one column for mass to charge ratio and added columns (Cell_Number,Ion_Mode,Before_or_After_PB).
* metadata included in the data file extracted to metadata.

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Data Files

File
mass_spec_double_bonds.csv (Comma Separated Values (.csv), 1.50 MB) MD5:53b963a7b8d807563b7c5b8b5ce9bf7c Primary data file for dataset ID 851911

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Related Publications

Benson, D. A., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J., & Wheeler, D. L. (2007). GenBank. Nucleic Acids Research, 36(Database), D25–D30. doi:10.1093/nar/gkm929

Methods

Liu, R., Zhang, G., Sun, M., Pan, X., & Yang, Z. (2019). Integrating a generalized data analysis workflow with the Single-probe mass spectrometry experiment for single cell metabolomics. Analytica Chimica Acta, 1064, 71–79. doi:10.1016/j.aca.2019.03.006
Methods

Pan, N., Rao, W., Kothapalli, N. R., Liu, R., Burgett, A. W. G., & Yang, Z. (2014). The Single-Probe: A Miniaturized Multifunctional Device for Single Cell Mass Spectrometry Analysis. Analytical Chemistry, 86(19), 9376–9380. doi:10.1021/ac5029038
Methods

Pan, N., Rao, W., Standke, S. J., & Yang, Z. (2016). Using Dicationic Ion-Pairing Compounds To Enhance the Single Cell Mass Spectrometry Analysis Using the Single-Probe: A Microscale Sampling and Ionization Device. Analytical Chemistry, 88(13), 6812–6819. doi:10.1021/acs.analchem.6b01284
Methods

Rao, W., Pan, N., & Yang, Z. (2016). Applications of the Single-probe: Mass Spectrometry Imaging and Single Cell Analysis under Ambient Conditions. Journal of Visualized Experiments, (112). doi:10.3791/53911
Methods

Smith, C. A., Maille, G. O., Want, E. J., Qin, C., Trauger, S. A., Brandon, T. R., … Siuzdak, G. (2005). METLIN. Therapeutic Drug Monitoring, 27(6), 747–751. doi:10.1097/01.ftd.0000179845.53213.39
Methods

Xia, J., & Wishart, D. S. (2016). Using MetaboAnalyst 3.0 for Comprehensive Metabolomics Data Analysis. Current Protocols in Bioinformatics, 55(1), 14.10.1–14.10.91. doi:10.1002/cpbi.11
Methods

Zhu, Y., Wang, W., & Yang, Z. (2020). Combining Mass Spectrometry with Paternò–Büchi Reaction to Determine Double-Bond Positions in Lipids at the Single-Cell Level. Analytical Chemistry, 92(16), 11380–11387. doi:10.1021/acs.analchem.0c02245
Results

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Parameters

Parameter	Description	Units
Cell_Number	Cell Number	unitless
Ion_Mode	Ion mode (Pos=positive ion mode, Neg=negative ion mode)	unitless
Before_or_After_PB	Description of whether before or after Paternò-Büchi (PB) reaction between unsaturated lipids (contain carbon-carbon double bonds) and acetone or benzophenone	unitless
Mass_to_Charge	Mass to charge ratio (m/z)	dimensionless

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Instruments

Dataset-specific Instrument Name	Thermo LTQ Orbitrap XL mass spectrometer (Thermo Scientific, Waltham, MA, United States)
Generic Instrument Name	Mass Spectrometer
Dataset-specific Description	Mass analyze parameters were as follows: mass resolution 60,000, 1 microscan, 100 ms max injection time, and automatic gain control on. A DC ionization voltage (+4 kV in the positive ion mode or −4 kV in the negative ion mode) was applied on a conductive union and transmitted through the solvent to induce ionization of cell lysis at the tip of the micropipette for MS analysis.
Generic Instrument Description	General term for instruments used to measure the mass-to-charge ratio of ions; generally used to find the composition of a sample by generating a mass spectrum representing the masses of sample components.

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Project Information

Collaborative Research: Creatine Cycling in Marine Bacterial and Phytoplankton Assemblages (Creatine Cycling)

Coverage: Atlantic bight

NSF Award Abstract:
High rates of dissolved organic nitrogen (DON) production and utilization in aquatic systems are typically attributed to microbial activity. Though it is known that there is a tight coupling between the production and consumption of biologically available DON, the composition, dynamics, and ecological significance of this rapidly cycled DON pool are less well understood. This proposal focuses on a component of the DON pool, creatine, which is historically understood as a product of metazoan activity, but appears to be both produced by phytoplankton and consumed by marine bacteria. Creatine is present in seawater in measurable quantities, which led to the hypothesis that creatine may be a significant component of the marine DON cycle. DON cycling likely has a bearing on fundamental marine ecosystem processes with large implications for carbon and nitrogen turnover on a global scale. Broader impacts of this project will include outreach that focuses on connecting scientists with K-12 students through research experiences for teachers and lesson development in collaboration with the K20 Center for Educational and Community Renewal, a statewide education research and development center at the University of Oklahoma. The project will integrate the research with inquiry-based teaching of rural secondary science teachers through Authentic Research Experiences in oceanographic science and microbial ecology. The K20 network includes 96% of Oklahoma schools, providing a unique opportunity to impact STEM education in Oklahoma.

The results of this project will help develop a better understanding of DON cycling, the ecological context of creatine uptake activity, and identify both creatine-producing and consuming organisms in the marine environment. The importance of creatine cycling will be assessed via 15N tracer studies along the natural coastal-to-offshore productivity gradient observed in the North Atlantic. Tracer and molecular approaches will be used to investigate the importance of phytoplankton vs. bacteria in creatine uptake and, the taxonomic identities of creatine-utilizing bacteria will be interrogated via molecular, stable isotope probing (SIP), and RT-qPCR approaches.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1634630

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