STABLE ISOTOPE PROBING OF COMPLEX LIPIDS THROUGH ISOTOPE PATTERN MATCHING OF MASS SPECTRA

J.S. Lipp[1], M. Könneke[1,2] and K.-U. Hinrichs[1]

[1] Organic Geochemistry Group, MARUM - Center for Marine Environmental Sciences & Dept. of Geosciences, University of Bremen, Bremen, Germany
[2] Marine Archaea Group, MARUM - Center for Marine Environmental Sciences, University of Bremen, Bremen, Germany

Stable isotope labeling experiments have provided a wealth of information on the metabolic traits of microorganisms and their turnover times in environmental samples (e.g., Takano et al., 2010; Wegener et al., 2016). Stable isotope ratios are typically determined using isotope ratio monitoring mass spectrometry (irmMS) on bulk samples through coupling to an elemental analyzer or on individual lipid compounds when coupled to gas chromatography (GC) (Hayes et al., 1990). The specific analysis of more complex lipids, which cannot be directly analyzed by GC, requires laborious extract fractionation followed by chemical degradation, e.g. saponification or ether cleavage to yield GC-amenable fatty acids or hydrocarbon chains, respectively (Kellermann et al., 2016).

The effect of stable isotope label incorporation during incubation experiments is easily visible in mass spectra when the label strength is high enough. Analytical protocols for proteomics routinely track uptake of isotope-labeled amino acids into peptides to study protein synthesis. However, similar protocols are only scarcely applied to the study of complex lipids and focused on the relative level of label incorporation into different compound groups under high labeling strength conditions (e.g., Fischer et al., 2013). We extended the approach to provide not only relative numbers but also accurate absolute isotope ratio values for both low compound concentrations and small label incorporation. Our algorithm uses the information encoded in high-resolution accurate mass spectra to directly compute stable isotope ratios for complex lipids separated by liquid chromatography (LC) without time-consuming wet chemistry procedures.

In order to validate the algorithm and provide a first-order approximation of analytical error we compared the new approach based on LC-MS to the gold standard of isotope ratio analysis, GC-irmMS. *Methanothermobacter thermoautotrophicus* (MTB) served as model organism, cultured either without addition of isotope label or with ^{13}C-labeled bicarbonate or ^{2}H-labelled water. Extracts of acid-hydrolyzed biomass were analyzed by LC coupled to high-resolution accurate mass time of flight (ToF) MS via an atmospheric pressure chemical ionization interface to yield mass spectra of archaeol and GDGT. The isotopologue patterns of GDGTs of theoretical and measured spectra were highly similar for the non-labeled culture when natural abundances were applied (~97% spectral similarity). Highest similarities of ~98% for the first three isotope peaks of GDGTs in the ^{13}C-labeled experiment were found for $\delta^{13}C$ values of 530‰ (Fig. 1). Notably, computed $\delta^{13}C$ values of archaeol were also around 550‰ and compared favorably to irmMS values of 530‰. The corresponding experiments with deuterium incorporation showed almost complete uptake into lipids. The analytical precision of the approach is limited by the accuracy of isotope pattern measurement in the mass spectrometer and is a function of label strength since the relative error in MS analysis of the isotopologue distribution directly propagates to a relative error in computed isotope ratios, i.e. higher values of isotope ratios of lipid analytes lead to higher uncertainties. We expect to reduce the currently observed relative error of ~15% determined

for ^{13}C and D for the studied compounds archaeol and GDGT by careful optimization of mass spectrometer and algorithm settings and subsequent design of a calibration method.

Our preliminary experiments demonstrate that isotopologue patterns of complex lipids can be exploited to directly compute isotope ratios. In stable isotope probing studies, this technique provides a straightforward, highly sensitive alternative to work-intensive GC-irmMS methods and is in principle applicable to stable isotopes of all elements found in lipids. This presentation will establish a comprehensive method validation and provide discussion of analytical and computational errors. Furthermore, it will be extended to other stable isotopes such as ^{15}N and ^{2}H and other compound groups including intact polar lipids, and different samples types such as incubation of environmental samples.
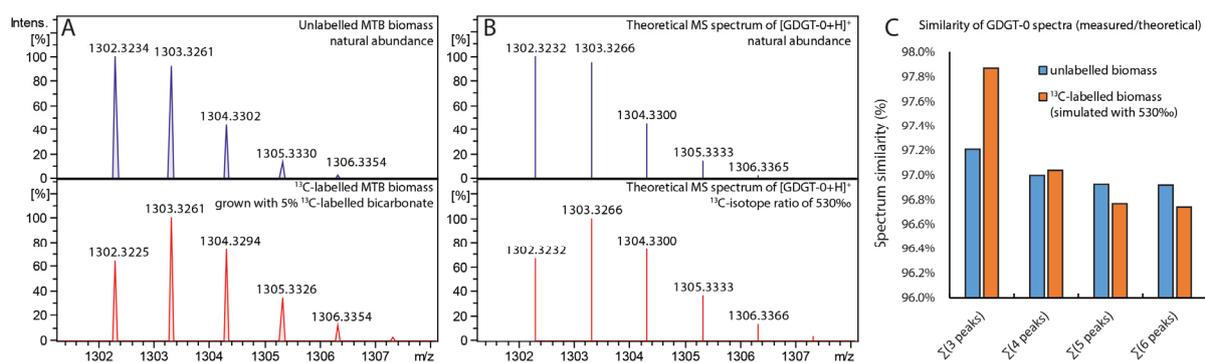

Figure 1: *Isotopologue pattern of (**A**) GDGT analyzed via LC-APCI-qToF-MS in cultures of Methanothermobacter thermoautotrophicus grown on bicarbonate as carbon source with natural abundance (top) and 5% label strength (bottom) and (**B**) computed theoretical isotope pattern of GDGT with natural abundance (top) and a δ^{13}C value of 530‰ which was determined by the least squares isotope pattern match (bottom). Panel (**C**) shows the spectrum similarity index calculated as "1 minus square root(sum of squared differences of each theoretical and measured peak abundance pair)" for non-labeled (blue) and ^{13}C-labelled (orange) MTB biomass. The inclusion of a higher number of isotopologue peaks is beneficial for larger molecules with a wider isotope pattern but leads to lower spectrum similarity indices and the potential to include signal spikes and thus needs to be evaluated carefully.*

References

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