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Tracking the signals of living archaea: A multiple reaction monitoring (MRM) method for detection of trace amounts of intact polar lipids from the natural environment

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ABSTRACT

Isoprenoid glycerol dialkyl glycerol tetraethers (iGDGTs) are biomarkers of archaea, but improvement is needed for more precise analysis of intact polar lipid (IPL)-iGDGTs. Here, we present a multiple reaction monitoring (MRM) method for IPL-iGDGTs using reversed phase liquid chromatography (RP-LC) and triple quadrupole mass spectrometry equipped with electrospray ionization (RP-LC–ESI-MRM). The MRM mode showed much greater sensitivity and lower detection limits than the commonly used selected ion monitoring (SIM) mode. Furthermore, the MRM mode had better stability and repeatability than the SIM mode. Thus, the RP-LC–ESI-MRM method can allow efficient fingerprinting of archaeal IPL-iGDGTs, particularly when they are in trace abundance in natural environments.

1. Introduction

Archaea have distinctive cell membranes that are commonly composed of isoprenoid glycerol dialkyl glycerol tetraethers (iGDGTs), which are ubiquitous in diverse environments (Schouten et al., 2013). Core lipid (CL)-iGDGTs are more stable and resistant to degradation, which permit them to serve as biomarkers of fossil archaeal lipids preserved in geological records. The polar head groups of intact polar lipid (IPL)-iGDGTs (Supplementary Fig. S1), especially phosphate-based groups, are more susceptible to degradation upon cell death (Zink et al., 2003). Therefore, IPL-iGDGTs can be used to estimate the living archaeal biomass in the natural environment (Pitcher et al., 2011).

Zhu et al. (2013) proposed a novel protocol coupling reversed phase liquid chromatography with mass spectrometry (RP-LC–ESI-MS) which enables the detection of ring and double bond distributions of IPL-iGDGTs (Zhu et al., 2013, 2014). However, the detection power of the full scan mode-based RP-LC–ESI-MS can be further improved by multiple reaction monitoring (MRM), an alternative detection mode widely used with gas chromatography–mass spectrometry (GC–MS) for analysis of biomarkers in trace amount (Summons, 1987). The MRM method applies tandem mass spectrometry (known as MS^2) to fragment pre-selected precursor ions. This technique effectively reduces coelution-derived noise that otherwise interferes with, or even overrides, the signatures of compounds of interest and therefore greatly improves their detectability. The MRM technique has been widely used to detect low/trace concentrations of organic molecules in proteomics analysis (reviewed by Domon and Aebersold, 2006) and co-eluted aliphatic biomarkers in petroleum geochemistry (Grosjean et al., 2009; Rohrssen et al., 2015). For the analysis of archaeal tetraether lipids, MRM or SRM (selected reaction monitoring) has also been employed to detect polar head groups of GDGTs in water column samples (Pitcher et al., 2011). However, SRM after normal phase LC (NP-LC–ESI-SRM; Pitcher et al., 2011) fails to detect the specific distribution of individual lipid homologues, and key ratios and indices cannot be measured, limiting its application for archaeal lipid-based geochemical studies.

We have extended the method of RP-LC–ESI-MS (Zhu et al., 2013) by combining RP-LC–ESI with the MRM technique. Compared with the widely used selected ion monitoring (SIM) mode, the MRM mode greatly lowers the detection limits and improves the repeatability for analysis of diverse IPL-iGDGTs in environmental samples.

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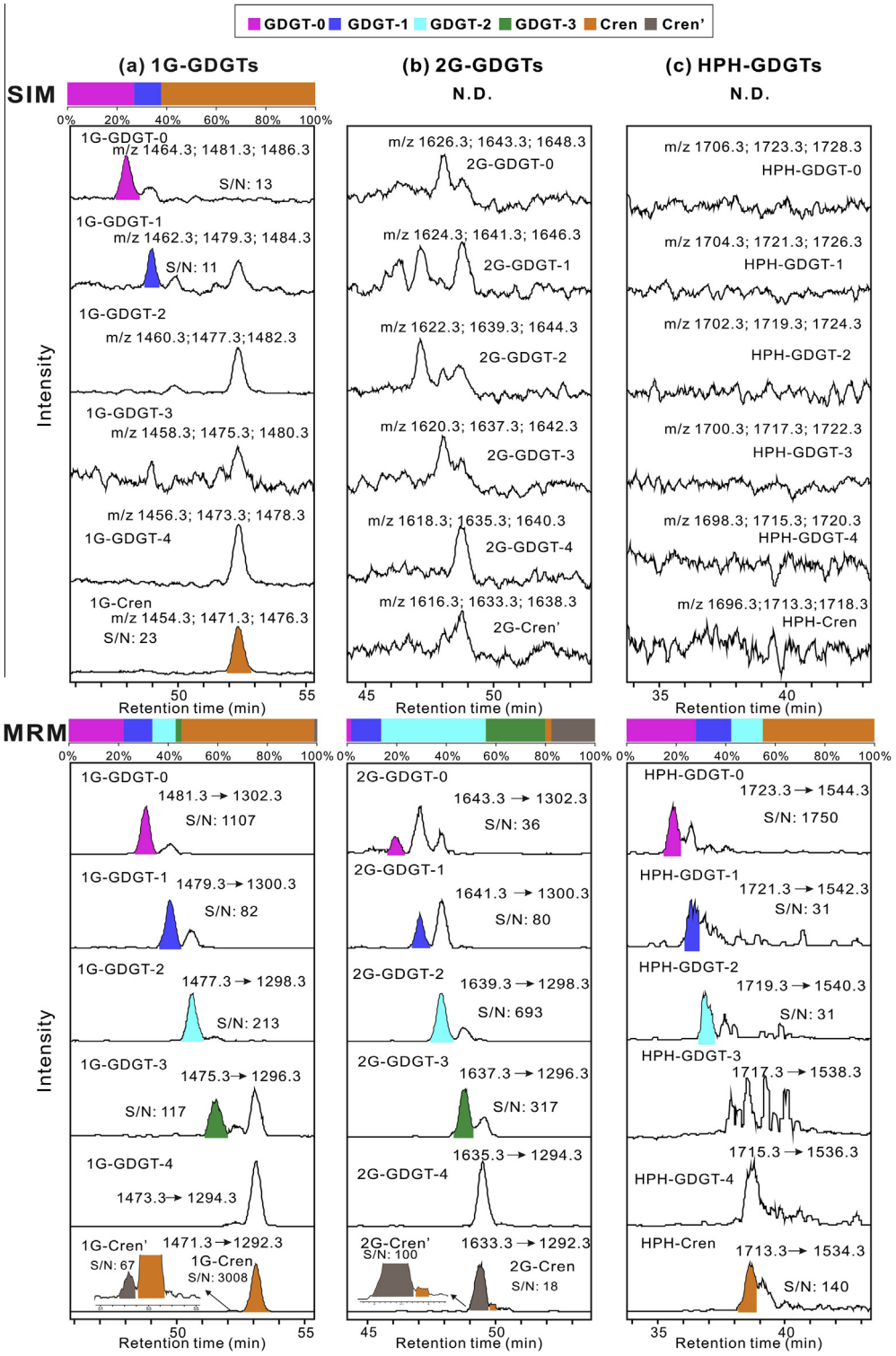

Fig. 1. Comparison of RP-LC–ESI-MS_{SIM} and RP-LC–ESI-MS_{MRM} for: (a) 1G-GDGT detection, (b) 2G-GDGT detection, and (c) HPH-GDGT detection with the same sample (SCS-sed). "→" represents the transition reaction in the MRM mode. IPL-iGDGT peaks with S/N > 5 were integrated.

Table 1
Parameters optimized for MRM method for specific IPL-iGDGTs. The fragmentor and cell accelerator voltage were 210 and 3.5 (arbitrary units), respectively. (CE: collision energy, arbitrary units).

Compounds	CE	Precursor ion $[M+NH_4]^+$	Product ion *m/z*	Compounds	CE	Precursor ion $[M+NH_4]^+$	Product ion *m/z*
1G-GDGTs	35	1481.3–1471.3	1302.3–1292.3	OH-GDGTs	20	1335.3–1331.3	1300.3–1296.3
2G-GDGTs	35	1643.3–1633.3	1302.3–1292.3	1G-OH-GDGTs	33	1497.3–1493.3	1300.3–1296.3
HPH-GDGTs	35	1723.3–1713.3	1544.3–1534.3	2G-OH-GDGTs	33	1659.3–1655.3	1300.3–1296.3

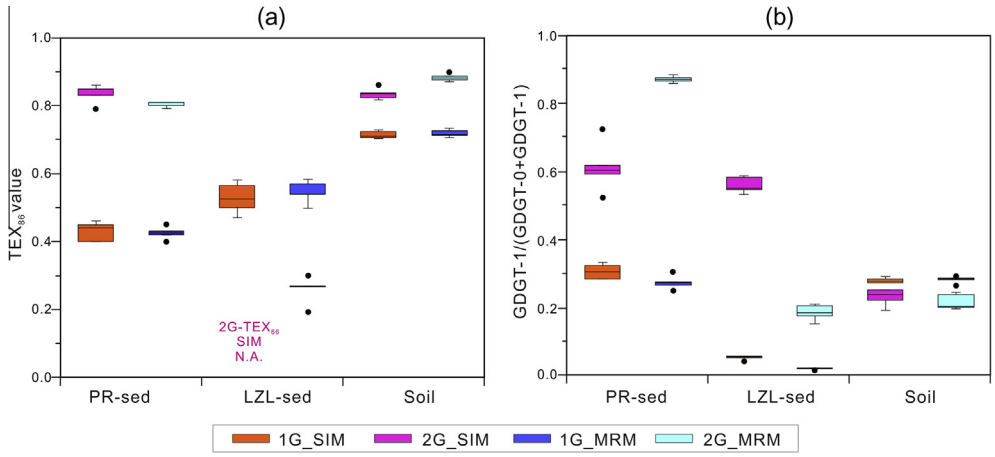

Fig. 2. Comparison of repeatability for (a) TEX_{86} value and (b) the ratio of GDGT-1/(GDGT-0 + GDGT-1) measurements under the SIM and MRM modes. The outliers were determined by the data that fell beyond the 1.5 × IQR (N.A. = not applicable). RP-sed = a sediment sample from Pearl River, LZL-sed = a sediment sample from Liangzi Lake, Soil = a soil sample from Yunnan (see Section 2).

2. Material and methods

Surface (0–5 cm) marine, riverine and lacustrine sediment samples were collected from the South China Sea (SCS-sed), Pearl River (PR-sed), Liangzi Lake (LZL-sed), respectively. A soil sample (Soil) from Yunnan, southwest China was also analyzed. Lipid extraction of the samples followed Sturt et al. (2004).

Analysis of total lipid extracts (TLEs) was conducted on an Agilent 1200 high-performance liquid chromatograph (HPLC) with an automatic injector coupled to a triple quadrupole 6460 MS equipped with an Agilent Jet Stream technology (CA, USA). HPLC conditions followed Zhu et al. (2013) with eluent A (MeOH:HCO_2H:14.8 M NH_3aq, 100:0.04:0.10, v/v/v) and eluent B (isopropanol:HCO_2H:14.8 M NH_3aq, 100:0.04:0.10, v/v/v). The separation was achieved with an ACE3 C_{18} column (3 μm, 2.1 × 150 mm) described in Zhu et al. (2013). β-L-Glucopyranosyl-caldarchaeti dyl-glycerol (> 95% pure, HPLC, Matreya LLC, PA, USA) was used as a representative of IPL-iGDGTs for ESI and MRM parameter optimization. The ESI source parameters were: gas 3 ml/min at 300 °C; sheath gas 9 ml/min at 300 °C; nebulizer 60 psi; capillary voltage 5 kV; nozzle voltage 700 V. The SIM and MRM were compared using the positive ion mode. In the SIM mode, the selected ions included protonated $[M+H]^+$, ammonium $[M+NH_4]^+$ and sodium $[M+Na]^+$ ions. In the MRM mode, only $[M+NH_4]^+$ ions were selected as the precursor ions. MRM parameters are displayed in Table 1. Data were analyzed with MassHunter Workstation Software Qualitative Analysis Version B.03.01 (Agilent Technologies Inc., USA). TEX_{86} values for 1G- and 2G-GDGTs were based on their GDGT core moiety distributions, respectively, as described by Schouten et al. (2002). In order to quantitatively compare the detection limits of RP-LC–ESI-MS_{SIM} and RP-LC–ESI-MS_{MRM}, analysis was performed on a dilution series of 1, 0.1, 0.01, 0.003 and 0.001 times the TLE abundance in sample PR-sed.

3. Results and discussion

We compared the detectability between SIM and MRM modes with the same TLEs (Fig. 1). Only 1G-GDGT-0, -1 and -Cren were shown clearly with a signal to noise ratio (S/N) > 5 under the SIM mode; whereas all 1G-GDGT compounds were clearly identified with strong peaks using the MRM mode (Fig. 1a); For 2G-GDGTs and HPH-GDGTs, the MRM mode showed significantly lower detection limits than the SIM mode (Fig. 1b and c). Hydroxyl core and intact polar (with hexose and dihexose head groups) GDGTs were also detected (Supplementary Fig. S2) showing better signals than the SIM mode. Three hexose GDGTs were well separated and clearly identified using the MRM protocol at the ×0.001 dilution. In contrast, 1G-GDGTs were not identified even at ×0.01 dilution of the TLEs in the SIM mode, demonstrating a 10 times higher detection limit than MRM (Supplementary Fig. S3).

The repeatability of the TEX_{86} measurement was evaluated by five injections of the same samples under the SIM and MRM modes with three representative samples (see Section 2). Generally, the average values of 1G-TEX_{86} were similar under the SIM and MRM modes in each type of the sample; however, a larger standard deviation (SD) was observed under the SIM mode (SD: 0.012–0.045) compared to the MRM mode (SD: 0.011–0.034) in different samples. The values of 2G-TEX_{86} were higher with larger deviations under the SIM mode (SD: 0.020–0.028) than that under MRM mode (SD: 0.009–0.043). Moreover, the 2G-TEX_{86} value was not applicable to the LZL-sed sample under the SIM mode because 2G-Cren′ was below the detection limit (Fig. 2a, Supplementary Table S1). Both methods showed similar values with similar SDs for the ratio of GDGT-1/(GDGT-0 + GDGT-1) for monohexose compounds in each type of the sample. The ratio values with dihexose head groups showed a much larger offset between the two methods, probably due to the interference of coelution-derived noise during the integration in the SIM mode. However, in terms of repeatability, the MRM mode still showed better SD (0.010–0.023) than that of the SIM mode (SD: 0.024–0.071; Fig. 2b, Supplementary Table S1).

4. Conclusions

Our study has demonstrated that RP-LC–ESI-MS coupled with MRM substantially lowers the detection limit and improves the repeatability compared with the widely used SIM mode. In particular, RP-LC–ESI-MS_{MRM} is useful for fingerprinting archaeal IPLs present in concentrations too low for SIM-based analysis.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.orggeochem.2016.04.006.

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References

Domon, B., Aebersold, R., 2006. Mass spectrometry and protein analysis. Science 312, 212–217.

Grosjean, E., Love, G.D., Stalvies, C., Fike, D.A., Summons, R.E., 2009. Origin of petroleum in the Neoproterozoic–Cambrian South Oman Salt Basin. Organic Geochemistry 40, 87–110.

Pitcher, A., Villanueva, L., Hopmans, E.C., Schouten, S., Reichart, G.-J., Sinninghe Damsté, J.S., 2011. Niche segregation of ammonia-oxidizing archaea and anammox bacteria in the Arabian Sea oxygen minimum zone. ISME Journal 5, 1896–1904.

Rohrssen, M., Gill, B.C., Love, G.D., 2015. Scarcity of the C_{30} sterane biomarker, 24-*n*-propylcholestane, in Lower Paleozoic marine paleoenvironments. Organic Geochemistry 80, 1–7.

Schouten, S., Hopmans, E.C., Schefuß, E., Sinninghe Damsté, J.S., 2002. Distributional variations in marine crenarchaeotal membrane lipids: a new tool for reconstructing ancient sea water temperatures? Earth and Planetary Science Letters 204, 265–274.

Schouten, S., Hopmans, E.C., Sinninghe Damsté, J.S., 2013. The organic geochemistry of glycerol dialkyl glycerol tetraether lipids: a review. Organic Geochemistry 54, 19–61.

Sturt, H.F., Summons, R.E., Smith, K., Elvert, M., Hinrichs, K.U., 2004. Intact polar membrane lipids in prokaryotes and sediments deciphered by high-performance liquid chromatography/electrospray ionization multistage mass spectrometry – new biomarkers for biogeochemistry and microbial ecology. Rapid Communications in Mass Spectrometry 18, 617–628.

Summons, R.E., 1987. Branched alkanes from ancient and modern sediments: isomer discrimination by GC/MS with multiple reaction monitoring. Organic Geochemistry 11, 281–289.

Zhu, C., Lipp, J.S., Wörmer, L., Becker, K.W., Schröder, J., Hinrichs, K.-U., 2013. Comprehensive glycerol ether lipid fingerprints through a novel reversed phase liquid chromatography–mass spectrometry protocol. Organic Geochemistry 65, 53–62.

Zhu, C., Yoshinaga, M.Y., Peters, C.A., Liu, X.L., Elvert, M., Hinrichs, K.-U., 2014. Identification and significance of unsaturated archaeal tetraether lipids in marine sediments. Rapid Communications in Mass Spectrometry 28, 1144–1152.

Zink, K.-G., Wilkes, H., Disko, U., Elvert, M., Horsfield, B., 2003. Intact phospholipids – microbial "life markers" in marine deep subsurface sediments. Organic Geochemistry 34, 755–769.