A protocol for simultaneous analysis of archaeal core and intact polar lipids using normal-phase HPLC/ESI-MS

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Isoprenoidal glycerol core lipids (CLs) from archaeal cell membranes have been utilized as proxies for paleo-environmental reconstruction (e.g., Schouten et al., 2002), and their intact forms (i.e., intact polar lipids: IPLs) can provide information on *in situ* archaeal activities in natural ecosystems (e.g., Lipp et al., 2008). Each form has been analyzed with normal phase (NP)-HPLC/ESI-MS for IPLs (Sturt et al., 2004) and with NP-HPLC/APCI-MS for CLs (Hopmans et al., 2000; Schouten et al., 2007) after the sample preparation (i.e. pre-separation of the total lipid extract into a polar and an apolar fraction, followed by acid hydrolysis of the polar fraction). However, such analytical protocols require relatively large amount of sample with great time and effort for the sample preparation. Furthermore, the acid hydrolysis of polar head groups and the APCI often cause degradation of labile lipids (e.g., hydroxyarchaeol) and byproduct formation (Liu et al., 2012; Sprott et al., 1990; Koga and Morii, 2005).

To overcome these shortcomings of traditional lipid analysis, we developed a novel NP-HPLC/ESI-MS protocol for simultaneous characterization of the distribution of CLs and IPLs from total lipid extracts in one analytical run. In order to cover the broad range of archaeal lipids in natural samples, the chromatographic separation and MS detection were optimized using the total lipid extracts of archaeal cultures having greatly different lipid compositions (*Methanosarcina barkeri* and *Sulfolobus acidcaldarius*).

With this protocol, CLs were separated according to structural variation of the alkyl chains and subsequently IPLs according to polar head group polarity into individual lipid classes. The distinct partitioning of separation part of CLs and IPLs is an important advantage when screening archaeal lipids in complex natural samples. Moreover, hydroxyarchaeol and their intact polar derivatives, which were lost in the conventional NP-HPLC/APCI-MS protocol, were detected in the total lipid extract from *M. bakeri*.

We also compared the quantitative accuracy and sensitivity on the detection of CLs between APCI and ESI-MS by using core GDGTs isolated and purified from a culture of *S.acidcaldarius*. As a result, the relative distributions of core GDGTs with NP-HPLC/ESI-MS were similar to those when analyzing with NP-HPLC/APCI-MS. This result suggests that our protocol can be applied to the study of core GDGT-based environmental proxies.

In addition to development of this protocol, we plan to apply the simultaneously chromatographic separation of this protocol to the preparation for compound-specific isotope analysis and structural identification of archaeal lipids, which will contribute for the study of archaeal activities in the environment that sample amounts are limited.

Keywords: Archaea, Intact polar lipids (IPLs), Core lipids (CLs), Simultaneous analysis

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