In order to investigate the microbial activities related to methane generation, we performed analyses of lipid biomarkers in the sediments from three wells (Alpha-1, Beta-1 and AT-1) in the eastern Nankai Trough by using the comprehensive two-dimensional gas chromatography (GC x GC). Previous geochemical studies have shown that the biogenic methane forms methane hydrate (MH) in the eastern Nankai Trough. Methanogens (methanogenic archaea) produce methane, which forms a vast quantity of methane hydrate in continental margin accretionary sediments. However, it is unclear at which depths methane was produced in the sediments. To address this issue, we attempted to identify and quantify the biomarkers of methanogens in the sediment cores by GC x GC equipped with qMS and FID.

The core samples at Alpha-1 and Beta-1 were collected from the eastern Nankai Trough by JOIDES Resolution during the multi-well drilling campaign "Tokai-oki to Kumano-nada" in 2004. Those at AT-1 was collected from Dai-ni Atsumi Knoll in the eastern Nankai Trough during site survey by the scientific drilling vessel CHIKYU in 2011. The lipids were extracted with methanol/dichloromethane, and the extract was saponified with 0.5 M KOH/methanol. The neutral fraction was converted to trimethylsilyl esters (TMS) by heating with BSTFA. The TMS-derivatives were analyzed using a ZOEX KT2006 comprehensive GC x GC equipped with qMS and FID. The content of total organic carbon and its isotopic ratio were determined by the flow-injection method using a Thermo DELTA V mass spectrometer connected with a Flash EA.

The neutral lipids fractions of the all core samples mainly consisted of n-alkanes, acyclic isoprenoids, n-alcohols, sterols and hopanols. Hopanols such as 17,21-homohopanol, 17,21-bishomohopanol, trishomohopane-32,33-diol and anhydrobacteri-ohopanetetrol were detected in all sediment samples, which might reflect the activity of in situ bacteria. The concentrations of hopanols in clay layers were significantly higher than those in sand layers. The TOC values were also higher in the clay layers. 2,6,10,15,19-Pentamethylicosane (PMI), which is considered to be the biomarker for methanogens and methanotrophic archaea, was detected in all samples from the three sites. Most of the delta 13C values of PMI were higher than -50 permil, suggesting that methanogens are the likely source organisms. In the sediments from Alpha-1 and Beta-1, PMI concentrations were relatively high at the MH bearing zone and below the MH bearing zone, suggesting higher abundance of methanogen biomass. In the sediments from AT-1, the concentrations were relatively high at 42mbsf and 216 mbsf. Interestingly, the methane production rates through the carbonate reduction pathway measured by 14C-tracer experiments were also high at these depths, exceeding 10 pmol/cm^2/d. Furthermore, hydrogenotrophic methanogens were also abundant at the same depths, as revealed by molecular analysis using deep rRNA gene sequencing. We therefore consider that PMI detected by GC x GC in this study is reliable as the indicator of methanogen biomass.

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