Coenzyme F430, quantification and isotope analysis from Hydrate Ridge California

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Coenzyme F430

Methyl-coenzyme M reductase, an enzyme traditionally associated with methanogenesis, has recently been linked to the anaerobic oxidation of methane. Coenzyme F430, a tetrapyrrole-nickel complex within the active site of methyl-coenzyme M, is used in methanogenesis and is hypothesized to play a key role in archaeal methanotrophy [1]. We adapted a method from Mayr *et al*.[2] to extract and isolate F430 from natural sediments so it can be purified for carbon and nitrogen isotope analysis. Isotope analysis is performed via a nano-scale elemental analyzer isotope ratio mass spectrometer (nano-EA-IRMS [3]).

Results

Here, we report F430 concentrations and isotopic data determined for sediment cores from active seeps at Hydrate Ridge (California), a well-studied site of anaerobic oxidation of methane (AOM). A spike in the concentration of F430 was observed at the 3-6 cm depth horizon of the core, which corresponds to peak abundance in ANME-2/*Desulfosarcina*/*Desulfococcus* aggregate counts. F430 was significantly more enriched in ^{13}C (-23‰ to -26‰) than hydroxy-archaeol isolated from the sediment (-60‰ to -120‰).

Our observations indicate F430 in this methanotrophic sediment is not derived from methane carbon, and potentially indicate methanotrophy does not take place via the reversal of methanogenesis. In contrast, the ^{13}C-depleted lipids and ^{13}C-enriched F430 signals reported here could also indicate both methane and inorganic carbon were assimilated by ANME.

Different carbon assimilation pathways during AOM have been previously suggested. Hallam *et al*.,[4] proposed carbon assimilation via the serine cycle or formaldehyde detoxification, as by methylotrophic bacteria. Bertram *et al*.,[5] reported evidence that ANME coproduced methane during methanotrophy, and that ANME can incorporate methane, acetate, methanol and bicarbonate carbon into lipid carbon. ANME AOM communities appear to have significant metabolic flexibility [5], which potentially accounts for the wide range of isotope signatures (~50 ‰) observed for AOM cell clusters in seep settings. We hypothesize assimilation of different carbon substrates by AMNE drive observed isotopic differences between Archeal lipids and F430.

[1] Scheller (2010) *Nature* **465** 606-609 [2] Mayr (2008) *J. Am Chem Soc* **130** 10758-10767 [3] Polissar (2009) *Anal. Chem.* **81** 755-763 [4] Hallam *et al* (2004) Science **305** 1457-1462 [5] Bertram *et al* (2013) *Environ. Microbio.* **15** 2384-2393