

Assessing the role of microorganisms in biogeochemical processes by protein immunodetection using nanoSIMS

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Anaerobic oxidation of methane (AOM) coupled to sulfate reduction, performed by consortia of methanotrophic archaea (ANME) and sulfate-reducing *Deltaproteobacteria*, plays a crucial role in biogeochemical carbon and sulfur cycling in marine sediments. We developed a method that allowed us to visualize single cells in AOM consortia, identify these cells based on the presence of key enzymes and determine their elemental and isotopic composition.

First, thin sections of AOM aggregates were prepared and examined by fluorescence microscopy. The discrimination between ANME and *Deltaproteobacteria* was achieved by using fluorescent antibodies targeting specific enzymes of each of the two organisms. Next, the sample preparation and immunolabeling protocol was successfully adapted for nanometer-scale secondary ion mass spectrometry (nanoSIMS). We selectively targeted the ANME with primary antibodies against methyl-CoM reductase – the key enzyme of AOM – and NanoGold-coupled secondary antibodies. The obtained nanoSIMS images revealed an unusually high phosphorus and iron content of the ANME-associated bacteria. The transmission electron microscopy coupled to energy-dispersive X-ray spectroscopy (TEM/EDX) showed that these bacterial cells contained peculiar amorphous iron- and phosphorus-rich particles in their cytoplasm.

Based on these results we speculate that AOM might also play a – yet unrecognized – role in iron and/or phosphorus cycling in marine sediments. This demonstrates that immunodetection of key enzymes in combination with nanoSIMS can be used to assess the involvement of functional groups of organisms in biogeochemical processes.

Reaction of silicate with released CO₂ by inorganic precipitations of marine carbonate in sandstone: Evidence from ⁸⁷Sr/⁸⁶Sr, δ¹⁸O and δ¹³C isotopes in calcareous sandstone

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⁸⁷Sr/⁸⁶Sr, δ¹⁸O and δ¹³C isotopes of carbonate in calcareous sandstone show intermediate values between marine carbonate and silicate phase of the sandstone.

Triassic Hiraiso Formation of the South Kitakami Terrane in Japan contains calcareous sandstone. The carbonate distributed homogeneously in lithic fragments and partly replaced plagioclase of the sandstone. It is considered that the early Triassic period was a dried climate and the Hiraiso Formation was deposited at near shore marine. The carbonate phase and silicate phase were separately examined. ⁸⁷Sr/⁸⁶Sr and δ¹⁸O isotopes of the carbonate phase are lower than the values of limestone at that Triassic period. The values show the intermediate between silicate minerals and marine carbonate, while δ¹³C shows the value of marine carbonate. The ⁸⁷Sr/⁸⁶Sr and δ¹⁸O isotopes indicate that these are the mixed ones of marine Sr and oxygen with those in silicate phase. Very few carbon is contained in the silicate phase and the δ¹³C value shows always that of marine. It shows *simultaneous* formations of the 1st: Carbonate precipitation from seawater in the sandstone with 2nd: Precipitation of carbonate by the quick reaction of silicates and the nascent carbon dioxide released at that time.