

Interactions Between Microbes and Dissolved Organic Matter at Deep-Sea Hydrothermal Vents

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The primary goal of this project supported by the Ocean Ridge Initiative was to characterize the composition of dissolved organic matter in high- and low-temperature hydrothermal vents in comparison to non-vent seawater. To address this goal, we modified an *in situ* pump to collect water from the seafloor. The system was then used to collect samples during an ROV *Jason* cruise led by Sievert to 9° North on the East Pacific Rise. The samples were collected from ambient seawater (2° Celsius), a high temperature hydrothermal vent (350° Celsius, Bio 9), and a low temperature vent (23° Celsius, Crab Spa). The dissolved organic matter was extracted from the water samples during the cruise and returned to WHOI for analysis by ultrahigh resolution mass spectrometry.

We used a two-pronged approach to analyze the composition of dissolved organic matter in these samples. Our targeted approach allowed us to quantify a pre-defined set of organic compounds. While this limited us to analysis of ~80 compounds, we achieved reliable quantitative information for each compound. We also conducted an untargeted analysis which provided a broader view of the organic compounds in our samples. While this approach was appealing because it did not require prior information about the organic compounds in our samples, it came at an added computational cost due to the challenges associated with the identification of organic molecules. For this project, the combination of the two methods allowed us to assess differences in the concentrations of specific molecules and to explore the compositions of organic molecules unique to deep-sea hydrothermal vents.

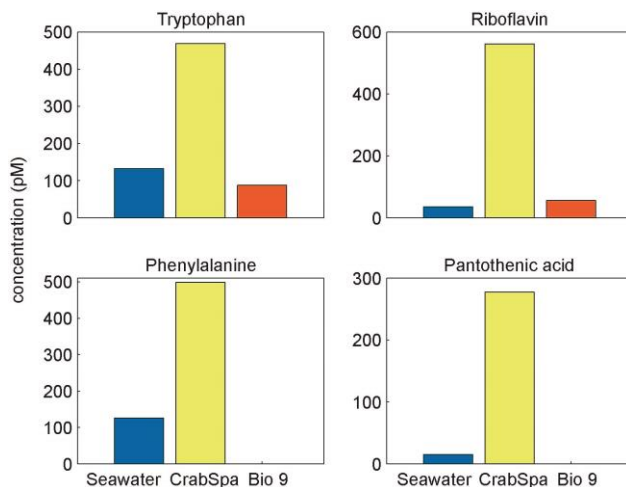


Figure 1: Most of the metabolites were present in the highest amounts in the Crab Spa sample. Shown here are concentrations of two amino acids (tryptophan and phenylalanine) and two vitamins (riboflavin and pantothenic acid).

The sample from Crab Spa (Figure 1) had the highest concentration of dissolved organic carbon and dissolved organic nitrogen. The Bio 9 sample had one-third of the dissolved organic carbon measured at Crab Spa and one-half of the dissolved organic nitrogen. The ambient seawater sample had concentrations of organic carbon and organic nitrogen consistent with non-hydrothermal vent deep-sea water indicating that the sampling



method designed for this project did not unintentionally add a high concentration of organic contaminants to the samples.

The compounds we measured with the targeted mass spectrometry approach are involved in central metabolic processes, and most of the measured compounds were found in the highest concentrations at the low temperature vent. In particular, the organic matter at the low temperature vent was dominated by water-soluble vitamins, amino acids, and nucleic acid precursors. For example, all of the metabolites seen in Figure 1 are present in higher concentrations at Crab Spa, but only tryptophan and riboflavin were also quantified from the high temperature vent sample. Note also that all of these compounds were observed in ambient seawater, but at lower concentrations.

Our untargeted assessment of the dissolved organic matter unique to the high and low temperature vents identified organic compounds with carbon-phosphorus bonds and peptides which were more prevalent in the high temperature vent fluid and absent from background seawater. The data in Figure 2 shows the peak height from the mass spectrometer for two of the unknown organic compounds putatively determined to have carbon-phosphorus bonds. Unknown compound #1 also contains nitrogen within the molecule. These molecules may represent energy and nutrient sources for microorganisms within hydrothermal vent fluids.

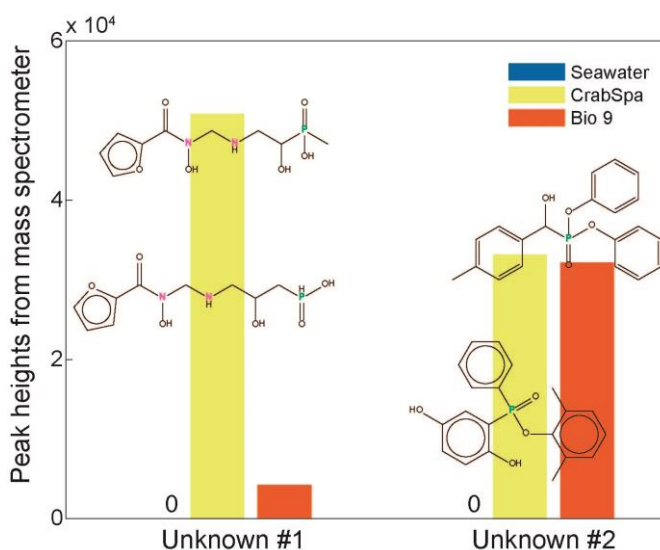


Figure 1: Example of two unknown compounds with possible chemical structures shown above each bar graph. Both of these compounds were absent from ambient seawater.

While these samples represent a limited dataset, they are the first molecular-level assessments of dissolved organic matter from deep-sea hydrothermal vents. Our results have indicated that the composition of dissolved organic matter at both low- and high-temperature vents includes compounds that are central to biological processes and provides insight into the composition of organic matter that fuels secondary, or heterotrophic, metabolic processes at deep-sea hydrothermal vents. These data are being developed into a manuscript (Longnecker, Sylva, Seewald, Sievert, and Kujawinski, "Biologically significant organic matter in high and low temperature hydrothermal fluids"). The data will also form the basis for a proposal to be submitted to NSF this year.

We are grateful for the support from the Ocean Ridge Initiative which we hope will leverage a larger grant to continue this research.

