Characterization of binding sites of metal ions at bacterial cell surface by REE distribution patterns and EXAFS analysis

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Interactions with bacteria can affect the migration of metal ions in natural waters through sorption and precipitation at the bacterial cell surface. In order to understand metal ion reactions with bacterial cell surfaces, it is essential to characterize the binding sites. Among various metal ions, rare earth elements (REE) are unique due to the systematic decrease of their ionic radii with the atomic number. Previous REE sorption studies with various bacterial strains [1, 2] showed an anomalous enrichment of heavy REE (HREE) to bacterial cell surfaces, which was likely related to the type binding site, since REE solid-water distribution patterns reflect the chemical characteristics of the adsorbents' binding sites.

The comparison of REE distribution patterns between water and bacteria to those between water and model compounds simulating bacterial binding sites (such as carboxymethyl cellulose, cellulose phosphate, alkylphosphate, peptidoglycan, and phospholipid) indicated that the HREE enrichment was caused by the binding to multidentate phosphate (phosphoryl) sites, while lighter REE were bound to phosphate (phosphoryl) sites with lesser coordination number.

The results from the REE patterns were complemented by EXAFS analysis at L_{III}-edges of various REE, such as Nd, Sm, Gd,, Tb, Ho, Er, Yb, and Lu. Heavier REE (Yb and Lu) onto bacteria and peptidoglycan exhibited similar structures to those complexed to alkylphosphate which forms multidentate phosphate complexes to REE. On the other hand, lighter REE (Nd and Sm) onto bacteria and peptidoglycan had similar structures to those sorbed on celluose phosphate. The coherent results of REE patterns and EXAFS show that two different phosphate sites are responsible for the sorption of REE, or other metal ions, onto bacteria.

[1] Takahashi *et al*. (2005) *Chem. Geol*. **219**, 53-67.
[2] Taksahashi *et al*. (2007) *Chem. Geol.* **244**, 569-583.

Methanogenesis at 122°C renews the upper temperature limit for life and the stable carbon isotopic fractionation of microbial methanogenesis

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Microbial methanogenesis in the deep-sea is a key process in the carbon cycle of Earth. It contributes to the CH_4 pool (free gas and methane hydrate) in deep-sea sediments, which is a potential energy source and alternative to petroleum as well as a strong greenhouse gas with a potential for rapid release. Hyperthermophilic methanogens are important primary producers in the hot ecosystem represented by the deep-sea hydrothermal areas and may represent the most ancient type of life flourishing in the early Earth. Nevertheless, the biogeochemical function and impact of methanogens in deep sea and deep subseafloor are poorly understood, in part because it is difficult to replicate the high temperatures and hydrostatic pressures in the laboratory. Here, we report a new technique for cultivation of chemolithoautotrophs including methanogens under high hydrostatic pressures. Using this technique, growth, survival and methane production of a newly isolated, hyperthermophilic methanogen *Methanopyrus kandleri* strain 116 was characterized under high temperatures and hydrostatic pressures. Elevated hydrostatic pressure extended the temperature maxium for growth from 116°C at 0.4 MPa to 122°C at 20 MPa, which exceeds the previously reported upper temperature limit for life of 121°C. In addition, piezophilic growth significantly affected stable carbon isotope fractionation of methanogenesis from CO_2. Under the conventional growth conditions, the isotope fractionation of methanogenesis by *M. kandleri* strain 116 was similar to values (ε = -30 ‰ to -25 ‰), previously reported for other hydrogenotrophic methanogens. However, under high hydrostatic pressures of 40 MPa, the isotope fractionation effect became much smaller (ε = -20 ‰ to >-15 ‰), and the kinetic isotope effect at 122°C was -13.1 ‰, which is the smallest effect ever reported. This observation will clarify the source of deep-sea methane as well as the ecophysiological and biogeochemical functions of methanogens and other chemolithoautrophs in deep-sea environments.