論文の内容の要旨

Biogeochemical Dynamics of Amino Acids in Marine Sediments: Constraints from Compound-Specific Nitrogen Isotopic Composition and D/L Ratio

（海洋堆積物中のアミノ酸の生物地球化学的動態：
化合物レベル窒素同位体組成と D/L 比からの制約）

山口　保彦

Organic matter (OM) in marine sediments is a major reservoir of organic carbon and nitrogen and plays an important role in the global biogeochemical cycles. Marine sediments also contain a vast amount of microbes. Interaction between sedimentary microbes and OM is an important process among various biogeochemical processes in marine sediments, because it has been suggested that sedimentary microbial ecosystems on most continental margins consist mainly of organic-fueled heterotrophs with relatively minor autotrophic components.

Amino acids are the building blocks of proteins and peptides and key compounds in microbial metabolisms. Amino acids represent one of major fractions of sedimentary OM and are important in undergoing OM mineralization in marine sediments. However, our understanding is still limited about the biogeochemical dynamics of amino acids in marine sediments, in part because currently available methods are not sufficient to constrain sources and transformation processes of amino acids in sediments (*e.g.*, composition analysis of amino acids, enantiomer ratio analysis of amino acids, isotope-labeling experiments).

The research objectives of this thesis are (i) to apply compound-specific nitrogen isotope composition of amino acids ($\delta^{15}N_{AA}$) at natural abundance as a new tool to estimate sources and transformation of amino acids in marine sediments and (ii) to constrain the biogeochemical dynamics (especially preservation and degradation mechanisms) of amino acids in marine sediments

using the $\delta^{15}N_{AA}$ method (combination with other chemical approaches). Towards the first objective (i), I investigated factors for controlling the $\delta^{15}N_{AA}$ signatures of microbes on culture experiments, which is essential to interpret $\delta^{15}N_{AA}$ data of environmental samples (Chapter 2). Next, as the second objective (ii), I analyzed the $\delta^{15}N_{AA}$ values of marine sediment core samples collected in two ocean regions (the Japan Sea in Chapter 3 and the Sagami Trough in Chapter 4). Furthermore, I proposed comparison of $\delta^{15}N$ of total hydrolysable amino acids (THAA) with $\delta^{15}N$ of other fractions (chlorin pigment in Chapter 3 and dissolved amino acids in Chapter 4) as a new, effective approach to constrain the biogeochemical dynamics of amino acids.

Nitrogen-isotope fractionation during amino-acids biosynthesis and decomposition by chemotrophic microbes (Chapter 2): $\delta^{15}N_{AA}$ has been demonstrated as a promising tool for estimating the food sources of organisms in the grazing food web. Utility of $\delta^{15}N_{AA}$ analysis to microbial processes in biogeochemistry of detritus organic matter remains uncertain, because the method has been constructed based on the analytical results of aquatic photoautotrophs (cyanobacteria and eukaryotic algae), terrestrial higher plants, and animals (aquatic and terrestrial), but not chemotrophic microbes. I analyzed $\delta^{15}N_{AA}$ in five cultured heterotrophic or chemoautotrophic microbes namely a fungus (*Saccaromyces cerevisiae*), a bacterium (*Escherichia coli*) and archaea (*Sulfolobus tokodaii*, *Halobacterium salinarum* and *Methanothermobacter thermautotrophicus*) with controlling their nitrogen sources. When the microbes synthesized amino acids *de novo*, the relative nitrogen-isotopic fractionation patterns of amino acids relative to glutamic acid ($\varepsilon_{x/Glu}$) were generally similar to aquatic photoautotrophs and terrestrial plants, with some exceptions such as phenylalanine of terrestrial C3 plants. Especially, the $\varepsilon_{x/Glu}$ values of the microbes (n=3) were very close to the aquatic photoautotrophs (n=37) for the most of amino acids such as alanine (0.2±2.2‰ and -0.1±2.2‰), isoleucine (-1.1±0.8‰ and -1.3±1.7‰), and phenylalanine (-3.0±1.0‰ and -2.7±2.2‰, respectively). In the case the microbes assimilated amino acids from the culture media, ^{15}N-enrichment factors of microbes relative to the media amino acids (*e.g.*, +8.2±0.8‰ for glutamic acid and +0.1±0.2‰ for phenylalanine, n=4) were close to animals (+8.0±1.1‰ and +0.4±0.4‰, respectively, n=11). These results suggest that similar processes control the nitrogen-isotope fractionation of amino acids in various organisms covering the three domains (Eukarya, Bacteria, and Archaea). Therefore, $\delta^{15}N_{AA}$ can be used as a powerful tool to clarify microbial metabolisms (*de novo* synthesis and decomposition of amino acids) and their biogeochemical roles in various environments including marine sediments.

Preservation mechanism of amino acids in marine sediments of the Japan Sea (Chapter 3): Degradation of amino acids does not achieve completely in surface sediments, although proteins and amino acids are considered to be biologically labile compounds. Several mechanisms have been discussed to explain the persistence of amino acids in subsurface marine sediments. Minerals or organic macromolecules may protect amino acids from microbial decay processes. It has been also

suggested that *in situ* production by sedimentary microbes would be an important mechanism for persistence of amino acids. Here, as a new method to estimate microbial alteration of amino acids in marine sediments, I analyzed compound-specific $\delta^{15}N$ of THAA in marine sediments of the Japan Sea (a surface sediment and a 7-m-long piston core; ca. 46,500 years). The down-core $\delta^{15}N$ profiles of THAA ($\delta^{15}N_{THAA}$) were compared with a down-core $\delta^{15}N$ profile of chlorin ($\delta^{15}N_{Chl}$), which reflects the $\delta^{15}N$ values of organic matter produced by photosynthetic organisms in the past ocean. Significant correlations were observed between the $\delta^{15}N_{THAA}$ and the $\delta^{15}N_{Chl}$ in the piston core samples (r^2 = 0.87 for phenylalanine (Phe), 0.78 for glutamic acid, 0.77 for alanine, and 0.62 for glycine; n = 13). This result suggests that the major source of THAA is organic matter produced by the organisms in the past ocean (*i.e.*, necromass) and that contribution of *in situ* sedimentary microbial production to THAA is less than 15% below 1 m depth in the core. The offset values between $\delta^{15}N_{THAA}$ and $\delta^{15}N_{Chl}$ in the sediments of 1-7 mbsf (*e.g.*, $\Delta\delta^{15}N_{Phe\text{-}Chl}$ = +7.3±1.0‰) suggest that the source organisms of THAA contain not only photosynthetic algae and animals but also heterotrophic or chemoautotrophic microbes in the past ocean (water column and surface sediments). These conclusions support the hypothesis that protection of amino acids from microbial decay processes is the important mechanism to explain persistence of amino acids in subsurface marine sediments. In addition, the significant correlations between $\delta^{15}N_{THAA}$ and $\delta^{15}N_{Chl}$ suggest a potential of $\delta^{15}N_{THAA}$ (especially phenylalanine) as a new paleoceanographic proxy of the nitrogen cycle in the past ocean.

Degradation mechanism of amino acids in deep-subsurface marine sediments of the Sagami Trough (Chapter 4): Although amino acids in sediment pore waters are key compounds in metabolic activities of sedimentary microbes and in mineralization of carbon and nitrogen, to date little is known about their biogeochemical dynamics (*e.g.*, sources and transformation processes) in deep-subsurface sediments. As a new approach to constrain the sources of dissolved amino acids in sediment pore waters, I analyzed and compared compound-specific $\delta^{15}N$ and enantiomer ratio (%D) of THAA in sediment solid phase and dissolved hydrolysable amino acids (DHAA) in sediment pore waters from the same sediment samples. I also conducted enantiomer-specific $\delta^{15}N$ analysis of alanine for THAA. Samples were collected from deep-subsurface sediments (up to 172.9 m below seafloor) at the Sagami Trough (NW Pacific). Compared to THAA in the same depth, DHAA show higher %D values in alanine, lower %D values in serine, similar %D values in valine, aspartic acid, glutamic acid, and phenylalanine ($\Delta\%D_{DHAA\text{-}THAA}$ = +15.0±2.0%, -7.2±6.7%, +2.8±6.1%, +0.2±2.6%, +3.4±4.0%, and -0.7±3.0%, respectively). In the sediments deeper than 9 mbsf, %D values of DHAA were 25.9±2.8% in alanine, 24.8±2.1% in aspartic acid, 11.3±2.8% in serine, and 16.3±2.7% in glutamic acid, and %D changes from THAA ($\Delta\%D_{DHAA\text{-}THAA}$) were +15.3±2.1% in alanine, -0.4±2.4% in aspartic acid, -8.1±6.2% in serine, and 4.6±3.3% in glutamic acid. Compound-specific $\delta^{15}N_{AA}$ analysis showed that $\delta^{15}N$ values of alanine are higher in the DHAA than

the THAA and that $\delta^{15}N$ values of glycine and glutamic acid are similar between the two fractions ($\Delta\delta^{15}N_{DHAA-THAA}$ = +5.8±2.3‰, +1.9±0.6‰, -0.3±1.1‰, respectively). Differences of $\delta^{15}N$ between D-alanine and L-alanine in the THAA fractions are significant only at two depths. These results suggest that the DHAA fractions have different $\delta^{15}N_{AA}$ and %D signatures from the THAA fractions, and that hydrolysis of the THAA is not the sole source of the DHAA. Alternatively, the $\delta^{15}N_{AA}$ and %D signatures of DHAA are consistent with the idea that *in situ* release of proteinaceous materials from sedimentary microbial biomass (such as peptidoglycan of Gram-positive bacteria) is an important source of DHAA. This suggests that recycle of dissolved amino acids by microbes would be an important process during amino-acids degradation in the deep-subsurface sediments.