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D-型と L-型のアミノ酸の同位体化学：生物起源と非生物起源のシグナル識別法
Enantiomer-specific isotope analysis (ESIA): D- and L-amino acids by biotic and abiotic processes

高野 淑識 [1*]
Yoshinori Takano[1*]

[1] 海洋研究開発機構
[1]JAMSTEC

Introduction

The one-handedness of terrestrial L-amino acids in proteins and in D-sugars of DNA and RNA are primary formation, structure and function of biopolymers for life on the Earth. Recently D-alanine has been recognized as a physiologically essential enantiomer for microbial growth and metabolic maintenance. The cell wall of domain Bacteria, especially for Gram-positive Bacteria, consists of a thick and uniform peptidoglycan layer that includes D-amino acids. Laboratory studies of the degradation of peptidoglycan showed it to decompose more slowly than proteins, indicating semi-labile compounds in nature. We have developed an analytical method to determine the ESIA of individual amino acid enantiomers and revealed nitrogen isotopic hetero- and homogeneity for D-alanine and L-alanine in terms of microbial processes in domain Bacteria and chemical processes in organic symmetric synthesis.

Experimental

The nitrogen isotopic composition of the individual amino acids was determined using a gas chromatograph/combustion/isotope ratio mass spectrometer (GC/C/IRMS) with a ThermoFinnigan Delta Plus XP combined with an Agilent Technologies 6890N GC and an Ultra-2 capillary column. Novel derivatization of amino acid diastereomers by optically active (R)-(-)-2-butanol or (S)-(+)-2-butanol with pivaloyl chloride produces N-pivaloyl-(R,S)-2-butyl esters (NP/2Bu) of the amino acid diastereomers. The elution order of these compounds on the chromatogram can be switched by a designated esterification reaction. We used purified peptidoglycans from domain Bacteria (phylum Firmicutes and Actinobacteria; Enterococcus faecalis, Staphylococcus aureus, Staphylococcus staphylolyticus, Lactobacillus acidophilus, Bacillus subtilis, Micrococcus luteus and Streptomyces sp.), (pseudo)-peptidoglycan from domain Archaea (Methanobacterium sp.), cell walls from domain Eukarya (Saccharomyces cerevisiae). Racemic D- and L-alanine were synthesized by a nucleophilic substitution 1 (SN1) reaction via an intermediate carbocation formed between α-bromopropionic acid (as amino acid racemic precursors) and aqueous ammonia.

Results and Discussion

The nitrogen isotopic difference of peptidoglycan defined as Δ 15ND-L in bacteria, representative gram-positive phylum Firmicutes and Actinobacteria, tended to be 15N-depleted in D-alanine, suggesting that heterogeneous components are mainly controlled by enzymatic pathways prior to formation of the bacterial cell wall. Alanine racemase (Enzyme Commission, EC; 5.1.1.1) that interconvert L-alanine to D-alanine, one of isomerases for chiral amino acids, previously indentified in a biosynthetic pathway, participates in crucial enzymatic reaction to form D-alanine before D-alanine-D-alanine ligase (EC; 6.3.2.4) pathway in peptidoglycan metabolism. In contrast, the Δ 15ND-L of racemic alanine in the chemical pathway during the nucleophilic substitution reaction between 2-bromopropionic acid and ammonia showed infinitely homogeneous components for each enantiomers. We present recent preliminary results in terms of abiotic geochemical samples for ESIA.

References

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Takano, Y., Chikaraishi, Y., Ogawa, O. N., Kitazato, H., and Ohkouchi, N. (2009) Compound-specific nitrogen isotope analysis of D-alanine, L-alanine, and valine: application of diastereomer separation to delta 15 N and microbial peptidoglycan studies. Analytical Chemistry, 81, 394-399.