Japan Geoscience Union Meeting 2013
(May 19-24 2013 at Makuhari, Chiba, Japan)

BAO01-P06 Room:Convention Hall Time:May 21 18:15-19:30

Enantiomer-specific isotope analysis (ESIA): D- and L-amino acids by biotic and abiotic processes

Yoshinori Takano[1*]

[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 alpha-bromopropionic acid (as amino acid racemic precursors) and aqueous ammonia.

Results and Discussion

The nitrogen isotopic difference of peptidoglycan defined as Delta15ND-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 Delta15ND-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

Takano, Y., Chikaraishi, Y. and Ohkouchi, N. (2010) Enantiomer-specific isotope analysis (ESIA) of D- and L-alanine: nitrogen isotopic hetero- and homogeneity by microbial process and chemical process. Earth, Life, and Isotopes. Kyoto University Press, pp. 387-402.

Chikaraishi Y., Takano Y., Ogawa O. N., and Ohkouchi, N. (2010) Instrumental optimization for compound-specific nitrogen isotope analysis of amino acids by gas chromatography/combustion/isotope ratio mass spectrometry. Earth, Life, and Isotopes. Kyoto University Press, pp. 365-386.

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.