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STABLE NITROGEN ISOTOPE ANALYSIS OF TETRAPYRROLES BY GAS CHROMATOGRAPHY/COMBUSTION/ISOTOPE RATIO MASS SPECTROMETRY

Yoshito CHIKARAISHI[1], Yuichiro KASHIYAMA[1], Nanako, O. OGAWA[1], Hiroshi KITAZATO[1], Shinya NOMOTO[2] and Naohiko OHKOUCHI[1]

1. Institute for Research on Earth Evolution, Japan Agency for Marine-Earth Science and Technology.
2. Department of Chemistry, University of Tsukuba.

Stable nitrogen isotopic signature of natural tetrapyrroles such as chloro- and pheo-pigments and alkylporphyrins is a potentially unique proxy for the reconstruction of nitrogen cycle in the aquatic surface environment in the modern to geological past. However, its application has been limited due to the analytical difficulties associated with these molecules. A significant problem is that nitrogen isotope analysis of tetrapyrroles cannot be carried out by gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS) due to low volatility and conjugated cyclic structures of tetrapyrroles, and that alternative isotope analysis by elemental analyzer/isotope ratio mass spectrometry (EA/IRMS) requires a considerable large amount of purified tetrapyrroles (more than micromolar amount of the elements) for the precise determination of isotopic compositions. Therefore, we developed a method to determine nitrogen isotopic composition of tetrapyrroles at nanomolar level, by a combination of chemical degradation treatment of tetrapyrroles into monopyrrole units (*i.e.* maleimides) and isotope analysis of the maleimides by GC/C/IRMS.

Two authentic (pyropheophorbide *a* and mesoporphyrin IX methylester) and four natural (chlorophyll *a*, deoxophylloerythroetioporphyrin (DPEP) and two 17-nor-DPEP) tetrapyrroles were demonstrated for the isotope measurements of this method. The chlorophyll *a* and DPEPs were isolated and purified from corn leaf and Miocene sedimentary rocks (Onnagawa Formation, Japan) by the improved procedures of Chikaraishi et al. (2005) and Kashiyama et al. (2007), respectively. These tetrapyrroles were degraded to maleimides by HCl treatment and chromic acid oxidation (*e.g.* Baker et al., 1968; Nomoto et al., 2001) prior to the isotope analysis.

For all authentic and natural tetrapyrroles, nitrogen isotopic composition of the observed maleimides was determined by GC/C/IRMS with a standard deviation (1σ) of better than ±0.5‰ at the minimum sample amount of 0.8 nmolN. The isotopic composition determined by GC/C/IRMS is consistent with that of original tetrapyrroles independently determined by EA/IRMS (Fig. 1). These results indicate that no substantial nitrogen isotopic fractionation occurs during the chemical degradation treatment, and that the isotopic

composition of tetrapyrroles can be accurately and precisely determined by the developed method at the sample amount as small as nanomolar level. This method is applicable for nitrogen isotope analysis of natural tetrapyrroles in various biological and geological samples.

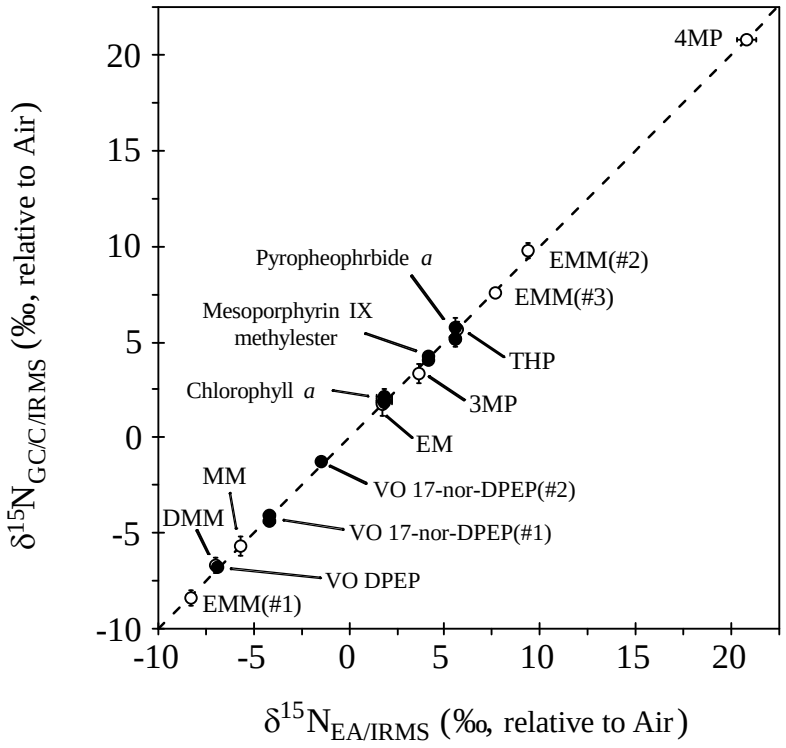

Figure 1. Comparison of nitrogen isotopic composition determined by GC/C/IRMS and EA/IRMS for synthesized maleimide standards (open symbol), and authentic and natural tetrapyrroles (filled symbol). Abbreviation: 2,3-dimethylmaleimide (DMM), 2-ethylmaleimide (EM), 2-ethyl-3-methylmaleimide (EMM), 2-methylmaleimide (MM), 3-Methylphtalimide (3MP), 4-methylphtalimide (4MP), 3,4,5,6-tetrahydrophtalimide (THP), deoxophylloerythroetioporphyrin complex to vanadium oxide (VO DPEP)

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