論文内容の要旨

論文題目：

Reconstruction of biogeochemical environment of the past ocean based on compound-specific carbon and nitrogen isotopic compositions of sedimentary porphyrins.

（化石ポルフィリンの分子レベル炭素・窒素同位体組成にもとづく古海洋環境復元）

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The present work established a novel methodology to reconstruct nitrogen and carbon isotopic compositions of the photoautotrophs of past environments by compound-specific isotopic analyses of sedimentary porphyrins, the chloropigments-derived organic molecules preserved in various geological samples. The work includes development of analytical methods for isolation and purification of individual sedimentary porphyrins, development of high-sensitivity techniques for determination of nitrogen and carbon isotopic compositions of isolated porphyrins, as well as elucidation of source-product relationships of each species of porphyrins. Altogether, it enables high-resolution studies of geological records on the population-specific reconstruction of photoautotrophic biochemistry, particularly the associated nitrogen cycling, ecological structure of the photoautotrophic community, and their paleoenvironmental conditions. It was then applied to organic-rich sediments with anaerobic/dysaerobic facies, namely (1) the diatomaceous siliceous rocks of Middle Miocene Onnagawa Formation, the record of a unique but regional anaerobic/dysaerobic setting and (2) the mid-Cretaceous Bonarelli black shale, the record of the global anoxic event, in order to elucidate the biogeochemistry of photoautotrophs and the paleoenvironment during formation of such peculiar geological records.

Theoretical backgrounds. Sedimentary porphyrins are extracted from various geological samples. Their common structure, the external five-membered ring, indicates that they have mostly derived from chloropigments of the past photoautotrophs (Fig. 1). They are structurally diverse, reflecting the original structures of the various precursory chloropigments. Because each variety of chloropigments is produced among specific groups of photoautotrophs, the origin of each species of sedimentary porphyrins is potentially narrowed to a particular population of photoautotrophs. On the other hand, consideration of biosynthetic and degradational reactions deduces that the nitrogen and carbon isotopic compositions of sedimentary porphyrin should constantly relate to those of the whole cell organic matters of photoautotrophs, which has indeed observed in the modern environments and cultures suggesting sedimentary porphyrins are approximately 4.8‰ depleted in ^{15}N and 1.8‰ enriched in ^{13}C. With this relationship, therefore, isotopic compositions of particular photoautotrophic populations are potentially reconstructed for the paleoenvironments.

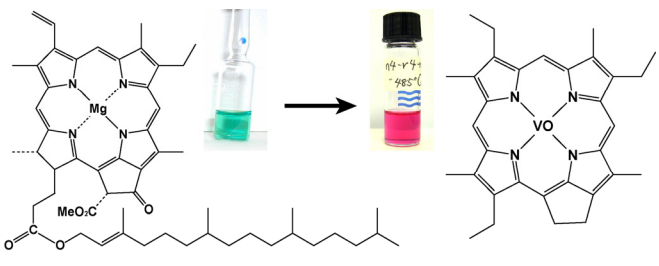

Figure 1: C_{32} DPEP (right), the most common sedimentary porphyrin, derives from chlorophyll *a* and other chloropigments

Nitrogen isotopic composition of marine photoautotrophs can be the proxy of nitrogen cycles associated to their photosynthetic activity. Specifically, it reflects what species of inorganic nitrogen is/was assimilated during their growth and also suggests the availability of these nutrients in the paleoenvironment. In particular, very characteristic nitrogen isotopic composition of -2 to 0‰ is expected for diazotrophic (i.e., N_2-fixing) photoautotrophs in a nitrate-depleted,

oligotrophic setting whereas a rather positive value is expected for nitrate-assimilating photoautotrophs in a marine environment such in a upwelling regions. Carbon isotopic composition of marine photoautotrophs reflects various factors including that of substrate CO_2 in the environment, sea surface temperature, growth rate, and the mode of CO_2 acquisition, that is, passive or active transport. In general, relatively negative value is likely in colder environment and/or slow-growing populations, whereas rapid growing community, particularly cyanobacteria and other groups when actively transporting the substrate, could result in a significantly elevated carbon isotopic composition.

Method developments. The development of the high-efficiency isolation/purification method for individual sedimentary porphyrin with high-performance liquid chromatography (HPLC), as well as preparation techniques for HPLC, was one of the most crucial steps of the present work. I herein proposed the dual-step HPLC method to purify various sedimentary porphyrins including nickel (Ni), copper (Cu), and vanadyl (VO) alkylporphyrins from the highly complex organic extracts of geological samples.

The high-sample-capacity reversed-phase HPLCs by adding N,N-dimethylformamide to the mobile phase allows an efficient collection of fractions containing the target compounds even using analytical-scale columns. Furthermore, this method achieved improved chromatographic resolutions but significantly reduced the overall retention time down to 60% compared to the previous work. The target compounds were then isolated with the normal-phase HPLC with the baseline resolution which is necessary to avoid chromatographic isotopic fractionation. One of the advantages of the method is that it requires neither derivatization nor demetallation. The purity of these isolated compounds was demonstrated by various HPLC online detection methods utilizing a photodiode-array detector, a mass selective detector to be better than 95‰ for 7 varieties of VO porphyrins, 12 varieties of Ni porphyrins, and 2 varieties of Cu porphyrins that were succeedingly analyzed isotopically. This HPLC method allows rapid and low-cost purifications using the analytical-scale columns. Therefore, it allows to apply isotopic studies of sedimentary porphyrins to various paleoenvironmental problems where handling a large number of samples is often required.

The nitrogen and carbon isotopic compositions were determined by an on-line system of isotope-ratio mass spectrometry coupled to an elemental analyzer (EA/IRMS) with improved sensitivity. The minimum quantity required for the determination of reliable nitrogen and carbon isotopic composition were successfully reduced down to approximately 3μg porphyrins, which is about two orders of magnitude smaller than the requirement in the previous work. Thus, the inclusive errors of the entire method that attributed to both instrumental conditions of EA/IRMS and potential artifacts during the purification procedure were estimated to be about 0.3‰ for both nitrogen and carbon (1σ).

Alternatively, purified porphyrins were derivatized into maleimides and analyzed for their nitrogen isotopic composition by gas chromatography/combustion/isotope-ratio mass spectrometry. This method allows determination of the confident isotopic value of nitrogen with the minimum requirement of approximately 140ng porphyrin with error of less than 0.5‰ (1σ), which is more than four orders of magnitude smaller than the previous work. This maleimide method was also applied to unresolved porphyrin fractions, by which nitrogen isotopic compositions of porphyrins having specific functions were still determined.

Evaluation of source-product relationships of sedimentary porphyrins. The specific origins of sedimentary porphyrin suggested by their structures were evaluated based on the nitrogen and carbon isotopic analyses. The relative isotopic variations among porphyrins with different molecular structures must indicate isotopic variation in their precursory pigments. Because the molecular structure suggests C_{32} deoxophylloerythroetioporphyrin (C_{32} DPEP; **1a**) to principally be derived from chlorophyll *a* that is synthesized by the majority of photoautotrophs, its isotopic composition is likely to represent the mean values of the entire photoautotrophic community. For this purpose, nitrogen and carbon isotopic compositions were determined for major species of VO and Ni porphyrins in multiple samples from the Miocene Onnagawa Formation and the mid-Cretaceous Livello Bonarelli black shale.

Unique biological origins were suggested for C_{30} 17-nor-DPEP (**1e**), C_{30} 8-nor-DPEP (**1d**), and C_{33} and C_{34} cycloheptanoDPEPs (**2a** and **2c**) of which isotopic compositions are significantly different from those of C_{32} DPEP (Fig. 2). Specifically, C_{30} 17-nor-DPEP was suggested to derive from chlorophylls *c* (**8a-c**), and their isotopic compositions must record those of chlorophylls *c*-producing algae, which represents majority of marine algae including diatoms, haptophytes, and dinoflagellates. C_{30} 8-nor-DPEP have probably originated from chlorophyll a_2, b_2, or c_2, or bacteriochlorophyll *a*.

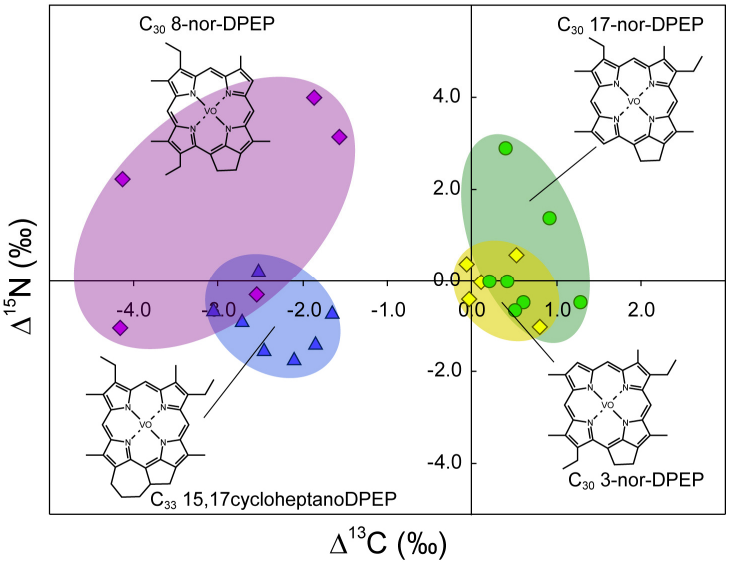

Figure 2: Diagram illustrating relative isotopic variations of various sedimentary porphyrins. $\Delta^{15}N$ and $\Delta^{13}C$ denotes the differences in nitrogen and carbon isotopic compositions of each species of porphyrins relative to C_{32} DPEP.

Although C_{33} cycloheptanoDPEPs, the probable products of herbivorous grazing of chlorophylls, are presumably derived from any biological sources as well, their isotopic compositions are distinctive to those of C_{32} DPEP (Fig. 2). This indicates that they represent a rather specific population of the photoautotrophic community. On the other hand, the isotopic compositions of one species of C_{32} ETIO-type porphyrin had been considerably different from those of C_{32} DPEP and any other porphyrins. It is thus most likely etioporphyrin III derived from heme, rather than chloropigments.

I conclude that C_{30} 17-nor-DPEP is reliable biomarker of the majority of marine algae; hence their isotopic composition records those of specifically algal population. However, certainly more investigations on isotopic compositions of sedimentary porphyrins, as well as those of early degradation products in the water columns and the surface sediment, are required for further exploring the potential of sedimentary porphyrins as paleoproxies.

Reconstructing photoautotrophic paleoecology/paleoenvironment for the organic-rich anaerobic sediments. Nitrogen isotopic compositions of VO C_{32} DPEP (-6.9 to -3.6‰; n = 7) from the Onnagawa Formation indicates that of the entire photoautotrophic community being approximately -2 to +1‰ (Fig. 3). Likewise, nitrogen isotopic compositions of Ni C_{32} DPEP (-6.6 to -4.8‰; n = 3) and Cu C_{32} DPEP (-5.7 to -5.1‰; n = 3) from the Livello Bonarelli black shale indicates that of the entire photoautotrophic community being approximately -2 to 0‰. These values strongly suggest for both examples that the nitrogen assimilated during phototrophic primary production was largely supplied *via* N_2-fixation by diazotrophic cyanobacteria. Moreover, nitrogen isotopic compositions of VO C_{30} 17-nor-DPEP (-7.4 to -2.4‰; n = 7) from the Onnagawa Formation indicates that marine algal population was also depleted in ^{15}N (-3 to +3‰). Thus, these algae should have been intimately associated with diazotrophic cyanobacteria that provided the most of inorganic nitrogen for the algal growth.

Carbon isotopic composition of VO C_{32} DPEP (-17.9 to -15.6‰; n = 7) and VO C_{30} 17-nor-DPEP (-17.2 to -15.1‰; n = 7) from the Onnagawa Formation indicates that of both the entire photoautotrophic community and the algal population being approximately -20 to -17‰ (Fig. 3). Likewise, carbon isotopic composition of Ni C_{32} DPEP (-20.5 to -17.9‰; n = 3) and Cu C_{32} DPEP (-20.1 to -16.3‰; n = 3) from the Livello Bonarelli black shale indicates that of the entire photoautotrophic community being approximately -22 to -18‰. Thus, the estimated isotopic effects associated with carbon fixation in the Onnagawa (-13 to -7‰) and the Bonarelli (-15 to -13‰) was strikingly small compared to those of the simulated *ordinary* photoautotrophic community in each paleoenvironments, namely, -20 to -14‰ and -23 to -20‰, respectively. The result suggests rapid growth rates for these photoautotrophs in an intense bloom conditions that perhaps had associated active transportation of carbon substrates and/or a significant rate of β-carboxylation. Again, these situations can be well explained if considerable contribution of cyanobacteria in the primary production.

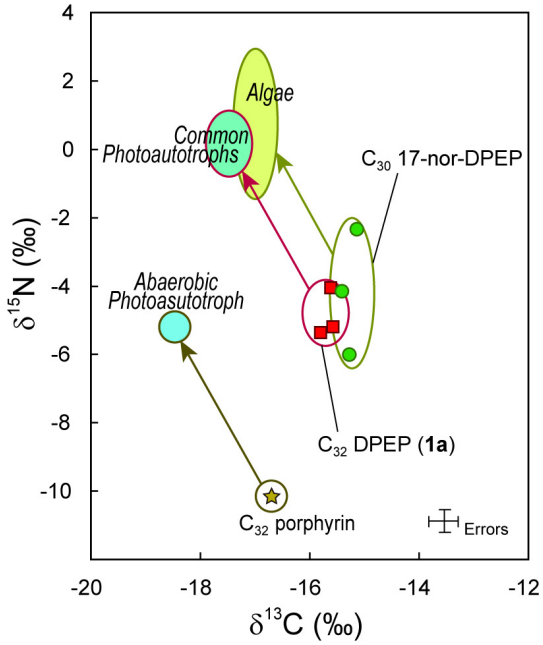

Figure 3: Some examples of reconstructed $\delta^{15}N$ and $\delta^{13}C$ values of photoautotrophic cells for samples from the Onnagawa Formation. Circles indicate approximate ranges of isotopic compositions for the photoautotrophic populations represented by each of individual porphyrin species.

Moreover, evidences of anaerobic photosynthetic activities were ubiquitous. All samples of both the Onnagawa Formation and the Livello Bonarelli black shale contained porphyrins with more than 34 carbon atoms that should have derived from bacteriochlorophylls *c~e* of the obligate anaerobic photoautotroph, green sulfur bacteria. In case of the Onnagawa, nitrogen isotopic compositions of maleimides derived from these porphyrins, as well as one species of C_{32} porphyrin, were significantly negative (-11.1 to -7.8‰), indicating the source organisms had assimilated ammonia in the reduced environment; hence confirming their origin of anaerobes. These thus suggest presence of reduced, anaerobic water mass within the photic zone (0-<200m) during the formation of both sedimentary records.

In conclusion, those two examples of organic-rich anaerobic sediments should have been formed under analogous paleoenvironmental conditions despite of their various sedimentological properties. Namely, these environments were commonly dominated by diazotrophic cyanobacteria in the primary production in presence of density stratified water column. Interestingly, these features are also common to the Mediterranean sapropels that formed intermittently during the Pliocene to the Holocene time. Thus, it is suggested that the prevailing oceanographic condition for the anaerobic sedimentary records in Phanerozoic oceans (i.e., under aerobic atmosphere) is anaerobic bottom water imposed by water column stratification rather than upwelling-driven elevated primary production. The dominance of cyanobacteria should be an inevitable consequence of water column stratification due to diminished supply of dissolved inorganic nitrogen to the surface water.

The rate of export production can be variable depending on the ecology of photoautotrophic community, that is, types of photoautotrophs responsible for the production, which should impose the lithological variation as well as variation in the sedimentation rate among anaerobic sediments. In particular, diatoms are important contributors in the primary production under cyanobacterial dominance. Diatoms in association to diazotrophic cyanobacteria, perhaps symbiotic cyanobacteria-hosted diatoms, were obviously important primary producers during the depositions of the Neogene examples of anaerobic sediments including the Onnagawa Formation and Mediterranean sapropels. On the other hand, diatom production is not obvious for the mid-Cretaceous Livello Bonarelli black shale. The difference may be the related to post-Cretaceous diversification of diatoms, particularly evolution of diatom-cyanobacteria symbiosis.