Stable carbon and nitrogen isotopic compositions of photosynthetic pigments as tracers for elemental cycle in the modern- and paleo-environments

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1. Introduction

Chloropigments drive photosynthesis, an essentially important process in the biogeochemical cycles on Earth. Eukaryotic phototrophs have chlorophylls (Chl) *a*, *b*, and *c*, while prokaryotic phototrophs except for cyanobacteria, have bacteriochlorophylls (BChl) *a*, *b*, *c*, *d*, *e*, and *g*. In this study we report carbon and nitrogen isotopic compositions of chloropigments in the saline meromictic Lake Kaiike [e.g., *Nakajima*, 2004; *Ohkouchi et al*., 2005]. Since Lake Kaiike is characterized by a dense chemocline population of photoautotrophic bacteria including purple sulfur bacteria (PSB), green sulfur bacteria (GSB), and cyanobacteria [*Nakajima et al*., 2003], it could be "model ocean" for oceanic environments during Cretaceous Oceanic Anoxic Events and Early Earth when the atmospheric oxygen level should have substantially lower than the present. Here, in conjunction with that molecular genetic information [*Koizumi et al*., 2004], we will discuss biogeochemical processes associated with species-independent microbial activities in this lake based on both carbon and nitrogen isotopic compositions of individual photosynthetic pigments. Furthermore, we will mention about the implication of the information for the studies of paleoenvironments.

2. Experimental

2.1 Samples

Lake Kaiike is located along the north shore of Kamikoshiki Island, southwest Japan. It has an area of 0.15 km^2 with a maximum water depth of about 12 m. Since it is located along the coast, seawater invades the lake through the bar to form a strong density stratification, resulting in a highly stable water column throughout the year. Therefore, it has a well-oxygenated surface water mass overlying an anoxic deep water mass. A dense population of bacteria has been observed around the O_2/H_2S interface. Based on the previous observations, the bacterial population is composed of two large-celled bacteria including purple sulfur bacteria and chemoautotrophic bacteria [*Matsuyama and Shirouzu*, 1978]. The cell number of each bacterium is up to 10^7 cells mL^{-1}. Water samples were collected with a convertible Niskin sampler near the deepest site of the lake. Portions of the water samples were filtered through GF/F filters to collect suspended particulate matter (SPM). Benthic microbial mats were collected by a SCUBA diver at the deepest site of the lake [*Oguri et al*., 2002]. Water properties of the lake were reported in *Nakajima et al*. [2003].

2.2 Analytical procedures

Photosynthetic pigments in filter and sediment samples from Lake Kaiike were extracted with acetone. Purification of the pigments was achieved by a two-step preparative high performance liquid chromatography (HPLC) method. The first step was rough purification by using a normal-phase HPLC. In this step, fractions containing isorenieratene, okenone plus a unidentified carotenoid, Pheo *a*, Chl *a*, BChl *a*, and BChls *e* plus BPheos *e* were isolated. The second step was achieved by a reverse-phase HPLC. In this step we purified individual BChl e homologues, okenone, isorenieratene, Chl *a*, Pheo *a*, BChl *a*, and BPheo *a*. The isolated pigments were judged to be free from impurities after evaluation based on both UV-Vis spectra and mass fragment in mass spectrometry. The purity of isolated chloropigments was also checked by performing elemental analysis. Measured C/N ratios of these isolated compounds were very close to theoretical ratios of the chloropigments, indicating that the purities are sufficiently high [*Ohkouchi et al*., 2005]. Pigment compounds were identified from UV-Vis spectra (350-850 nm) and mass spectra of atmospheric pressure chemical ionization-mass spectrometry. Quantification of pigments was achieved by UV-Vis absorbance. The detector response factors of chloropigments were determined by linear regressions of replicate analyses for known amounts of standard solutions [*Nakajima et al*., 2003].

Carbon and nitrogen isotopic compositions of these samples were determined by an on-line system of Finnigan Delta Plus XP mass spectrometry connected with an elemental analyzer (Flash EA1112) through a Conflo III interface. The isotopic compositions are presented as conventional δ notations; $\delta X = (R_{sample}/R_{standard} - 1) \times 1000$ (‰), where X is ^{13}C or ^{15}N and R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Standards are PDB for carbon and atmospheric N_2 for nitrogen. Based on the replicate runs of authentic and laboratory standards, the analytical error (95% probability) was estimated to be better than 0.2‰ for both isotopic compositions.

3. Lake Kaiike; a case study in a saline meromictic lake

In the chemocline, BChl a related to the PSB (*Halochromatium sp*.) have $\delta^{13}C$ values ~-31‰, whereas BChls e related to the GSB are ~-22‰ (Fig. 1). Since the isotopic composition of dissolved inorganic carbon (DIC) in this depth is -2.1‰, the apparent isotopic discriminations between DIC and chloropigments are -26.6‰ for Chl *a*, -28.9‰ for BChl *a*, and -20.3‰ for BChls *e*. The relatively small isotopic discrimination of BChls e could be ascribed to the fact that the GSB fix CO_2 through a reverse TCA cycle [*Holo and Sirevåg*, 1986] which has an isotopic fractionation ~10‰ smaller than that of the Calvin cycle as utilized by PSB and

cyanobacteria [e.g., *Quandt et al*., 1977]. The BChls *e* at 8 m are 1-2‰ depleted and those from microbial mats are 7‰ depleted in ^{13}C relative to those from the chemocline. It suggests that the low-light adapted GSB inhabit the monimolimnion and microbial mats with producing substantial amount of BChls *e* by assimilating ^{13}C-depleted regenerated DIC. By contrast, both BChl *a* from the PSB have isotopic compositions around -31‰ with small variations with depth (Fig. 1). Since the PSB inhabit no more than 1 m thickness in the upper portion of the anoxic zone [*Matsuyama*, 2002], the BChl *a* in 8 m and surface sediments should originally be formed in the chemocline. This is consistent with the genetic evidence indicating that the PSB-related DNA is observed only in the chemocline [*Koizumi et al*., 2004]. At 3 m, the $\delta^{13}C$ values of Chl *a* is -24.4‰, whereas in the chemocline it is -28.7‰ (Fig. 1). In the monimolimnion cyanobacterial DNA fragments were little found in the PCR-DGGE band analyses [*Koizumi et al*., 2004]. Furthermore, at 8 m Chl *a* is somewhat enriched rather than depleted in ^{13}C relative to that of the chemocline. These lines of evidences suggest that the activity of cyanobacteria in the monimolimnion is negligibly small, and the Chl *a* in 8 m could be a mixture of Chl *a* originally formed by diatoms in the oxic mixolimnion and by cyanobacteria in the chemocline. In the benthic microbial mats, the Chl *a* is again more enriched in ^{13}C relative to the chemocline. Therefore, we conclude that the cyanobacterial activity is also negligibly small in the microbial mats.

The $\delta^{15}N$ values of BChl *e* homologues are 3-4‰ depleted in ^{15}N relative to those in the microbial mats (Fig. 2). The different nitrogen isotopic signatures support above interpretations that the GSB inhabit the benthic microbial mats and synthesize BChls *e*. The $\delta^{15}N$ of BChl *a* in the chemocline is -2.1‰, which is substantially heavier than those of BChls *e*. Several studies have reported that the Chl *a* is 5 to 7‰ depleted in ^{15}N relative to the biomass [*Sachs et al*., 1999; *Beaumont et al*., 2000]. Such a ^{15}N-depletion in the Chl *a* has been ascribed either to isotope effects associated with transamination of glutamic acid to form aminolevulinic acid, to physiological factors related to growth rate, or to different nutrient sources. Assuming that the isotopic discrimination between chloropigments and biomass is 6‰, mean $\delta^{15}N$ values of GSB and PSB in the chemocline are estimated to be *ca*. -1 and 4‰, respectively. We think that the $\delta^{15}N$ value for the GSB assimilated nitrogen through the N_2 fixation pathway, which produces organic matter 0-2‰ depleted in ^{15}N relative to the substrate [e.g., *Wada*, 1980]. If the GSB uptake ammonium, the isotopic fractionation associated with the process should be about -15‰, a value substantially smaller than those estimated before [e.g., *Pennock et al*., 1996]. However, physiological studies have suggested that the N_2 fixation processes in the aquatic environments "switch off" where ammonium is present [e.g., *Madigan*, 1995]. Hence, the consideration based on the $\delta^{15}N$ evidence appears to contradict physiological knowledge at this moment and further studies are required for solve this problem. The concentration of dissolved ammonium in the chemocline (26 mM!) might be too small to switch off the activity of nitrogenase in *C. phaeovibrioides*. Alternatively, a part of GSB may utilize ammonium in the chemocline. Based on the reconstructed $\delta^{15}N$ value of PSB, they apparently do not conduct N_2 fixation. We also do not think that the PSB utilize ammonium, because the apparent isotopic fractionation (ca. -9‰) is substantially smaller than those reported previously [e.g., *Fogel and Cifuentes*, 1993]. Therefore, nitrite may be a potential nitrogen source for PSB and cyanobacteria inhabiting the chemocline.

4. Implications for paleoceanography

It has been known that the porphyrin nucleus of the chloropigments is very stable during the diagenetic and catagenetic processes. They have been found even in the petroleum which experienced high P-T conditions. Since they carry the isotopic information of carbon, nitrogen, and hydrogen, the porphyrins are potentially exellent tools as biogeochemical tracers applicable to wide range of geological samples. So far, several studies have discussed carbon cycle in the geological past based on the carbon isotopic composition of "geoporphyrins" [e.g., *Hayes et al*., 1987; *Popp et al*., 1989]. However, few studies have reported nitrogen isotopic composition of geoporphyrins due to the analytical difficulty concerning purification of large amount of geoporphyrins which is required for the determination of isotopic compositions [*Chicarelli et al*., 1995]. Currently, we are developing an analytical methods to purify the geoporphyrins from the sediments by using a preparative-HPLC and determine the nitrogen isotopic composition of these porphyrins with as small as <5 mgN by using both elemental analyzer/isotope ratio mass spectrometry. By using the improved system, we have recently obtained preliminary results from Cretaceous black shales, which suggest that nitrogen fixation is a major pathway to assimilate nitrogen by primary producers during the Oceanic Anoxic Events [*Kashiyama et al*., in prep.]. In near future, the high-throughput, robust analytical methods for determination of isotopic compositions of geoporphyrins will be established, which potentially contribute to the precise reconstruction of the oceanic environments in the geological past.

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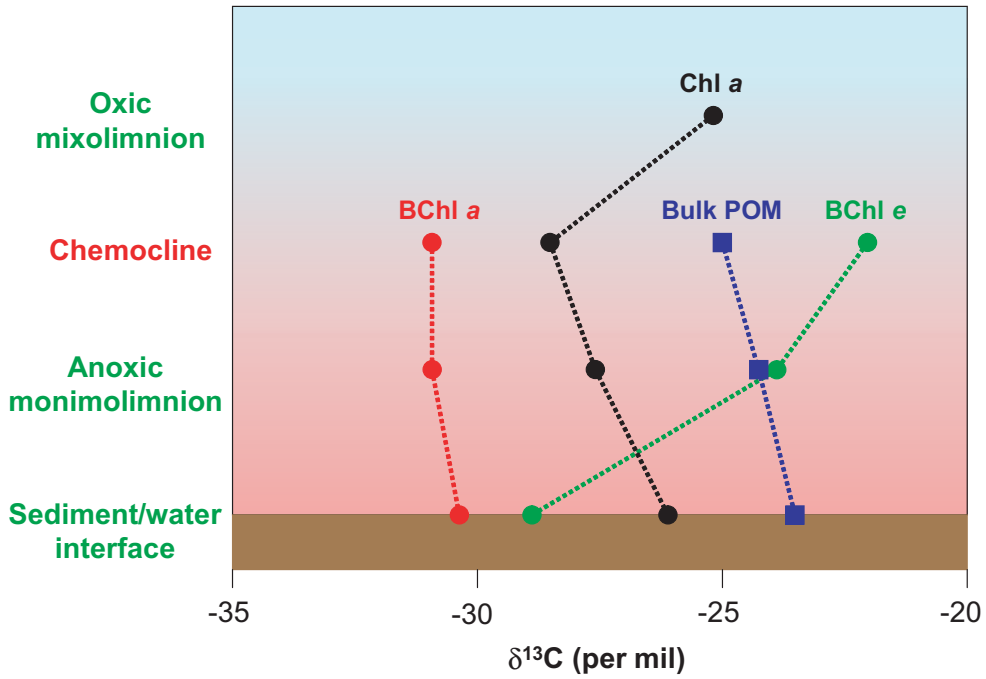

Figure 1. Distribution of carbon isotopic compositions ($\delta^{13}C$) of chloropigments and bulk particulate organic matter (POM) isolated from Lake Kaiike.

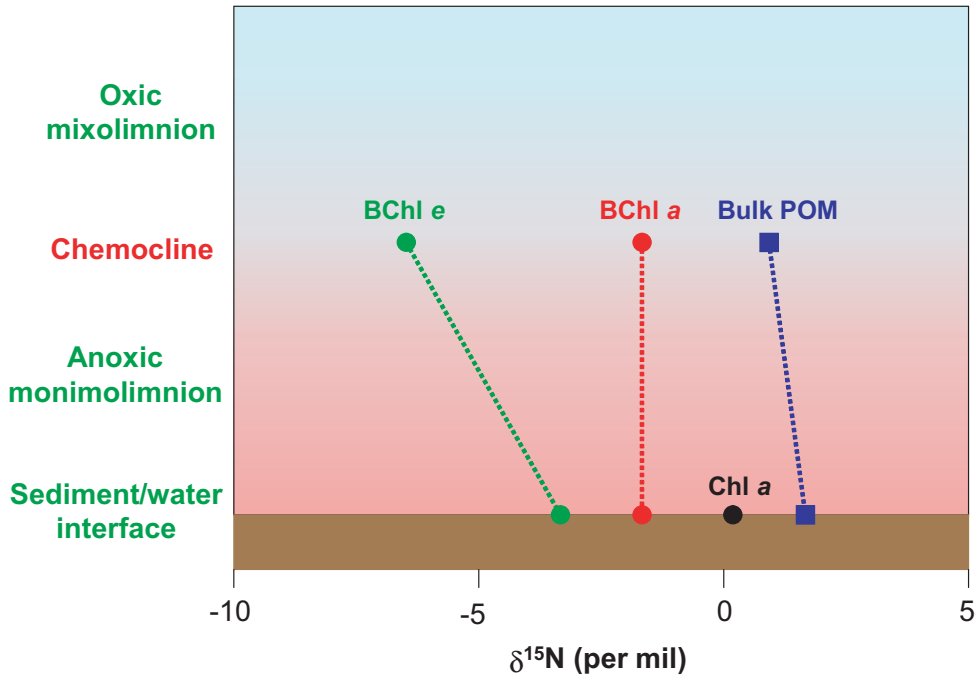

Figure 2. Distribution of narbon isotopic compositions ($\delta^{15}N$) of chloropigments and bulk particulate organic matter (POM) isolated from Lake Kaiike.