HEME AS ONE OF THE KEY MOLECULES FOR CONSTRAINING MARINE BIOGEOCHEMICAL CYCLE

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Introduction
Hemes are Fe(II)-tetapyrrole complexes possessed by all life forms, catalyzing various electron transfer and redox reactions essential for biological systems. As they represent ~40% of the iron pool in phytoplankton (Gledhill, 2007), heme-iron in the marine biomass likely constitutes a major fraction in the bioavailable-iron pool which limits primary productivity in over 30% of the world oceans (Fig. 1a). Indeed, phytoplankton and bacteria have shown to directly uptake heme from the environment (Hutchins et al., 1999; Hopkinson et al., 2008). Distributions of heme in the ocean have been investigated in several previous studies, reporting up to 20 pmol L⁻¹ of heme b, a major homolog of hemes, in particulate material (> 0.7 µm: Gledhill et al., 2013; Honey et al., 2013). However, more detailed analyses are necessary to understand its functions in the marine iron cycle. Here, we have established a method for quantification and determination of both carbon and nitrogen isotopic compositions (δ¹³C, δ¹⁵N) of heme b, in an attempt to constrain their sources and dynamics in the ocean.

Results and Discussion
Our new analytical method consists of multi-step extraction, ion-exchange open column chromatography, and dual-step preparative HPLC, which enable us to quantify, isolate, and purify heme b from various environmental samples (Fig. 1b). Detection of picomole levels of heme b is achieved with a photodiode-array detector connected to HPLC, covering the concentration range of heme b in particulate and dissolved organic matter of coastal seawater. The δ¹³C and δ¹⁵N values of the purified heme b are determined with our modified EA/IRMS (Ogawa et al., 2010). Measured C/N ratio and LC/MS spectra of heme b in native and methyl-esterified forms confirm that they are well-purified from various environmental samples.

Amount of heme b in particulate material collected from the coastal seawater of Tokyo Bay (> 20 µm; 2.7–15.3 µg g⁻¹ dry weight) were comparable to those of biological samples (e.g., diatom, cyanobacteria; 0.7–18.2 µg g⁻¹ dry weight), and their concentrations were as high as 0.15 nmol L⁻¹ which are equivalent to ~15% of particulate iron concentration in typical coastal seawater (Johnson et al., 1997). Interestingly, heme b concentration in the dissolved phase was contrastingly low (> 3000 Da ultrafiltration; ~0.006 nmol L⁻¹), corresponding to only a few percent of typical dissolved iron concentration. These results imply that heme in particulate material may represent a major component of the regenerated iron (Fig. 1a), which is not well constrained but may account for as much as 50–90% of the total iron supply to the photic zone (Boyd et al., 2010). Heme b purified from marine biological samples and commercially-available hemoglobin standard showed varying δ¹³C and δ¹⁵N values (Fig. 1c). These isotopic compositions will be compared with those of amino acids and chlorophylls to constrain the isotopic fractionation associated with the heme biosynthesis, and to determine its sources in marine environments. Such insights are also essential in revealing the origin of etioporphyrin III preserved in various sedimentary rocks (Fig. 1a), which has a highly distinct isotopic signal with respect to other porphyrins derived from chlorophylls in Cretaceous black shales (Ohkouchi et al., unpub. results). Detectable amount of heme b was indeed found in surface sediments of the Japan Sea (~0.03 µg g⁻¹ dry weight). Our new method will provide first
detailed geochemical insights into organic iron-binding ligands playing an essential role in marine biogeochemical cycle. We are also currently establishing a method to determine $\delta^{56}$Fe of heme, which likely records information on the source iron assimilated by the organisms.

**Figure 1** (a) Schematic diagram of the marine iron cycle. Heme may comprise a major regenerated iron compound supplied to organisms. They may be degraded into etioporphyrin III in the sediments. (b) Reversed-phase HPLC chromatogram of the marine POM sample. (c) Carbon and nitrogen isotopic compositions of heme $b$ purified from various samples.

**References**


