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Iron isotope fractionation during uptake of ferrous ion by phytoplankton

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ABSTRACT

The uptake of iron (Fe) by phytoplankton is an important pathway that drives the global Fe biogeochemical cycle. However, limited information is available regarding the resulting Fe isotope signatures during this metabolic processes. Here, two algal species *Chlorella pyrenoidosa* and *Chlamydomonas reinhardtii* were cultured in a medium spiked with $FeSO_4$ to study their ability to fractionate Fe isotopes. We quantified the total cellular and intracellular Fe, and measured their isotope compositions. The amounts and isotope compositions of extracellular Fe were estimated by mass balance. We found that the intracellular Fe of algae in concentration-gradient experiments was enriched in the heavier isotopes relative to the $FeSO_4$ solutions, up to 3‰ in $\delta^{56}Fe$, suggesting the heavier Fe isotopes are preferably taken up by the algae. However, the intracellular Fe of algae in time-course experiments showed inconsistent fractionation patterns, either enriching or depleting heavier Fe isotopes. Extracellular Fe was isotopically variable from −2.5‰ to 1.9‰ in $\delta^{56}Fe$ relative to the $FeSO_4$ solutions, likely representing a mixture of Fe^{II} and Fe^{III} adsorbed on the cell surface. Additionally, the variation of intracellular $\delta^{56}Fe$ values appears to be dependent of the intracellular Fe fractions, enriching heavier Fe isotopes at the lower intracellular Fe fractions. Our observations not only highlight the potential of using Fe isotopes as the tracer of biological Fe cycles, but also have important implications on the Fe metabolic pathways of algae.

1. Introduction

Iron (Fe) is a redox-sensitive element, and mainly exists as reduced ferrous Fe (Fe^{II}) in oxygen-poor environments and oxidized ferric Fe (Fe^{III}) in oxygen-rich environments. However, the availability of Fe in the oxygenated waters is generally low due to the low solubility of Fe^{III}. As a micronutrient element, Fe is vital for biological activities, and is considered as a limiting factor of phytoplankton growth in high-nutrient, low chlorophyll regions of the ocean (Clasen and Bernhardt 1974; Coale et al. 1996; Martin et al. 1994). The Fe uptake process during phytoplankton metabolism is crucial to understand the global biogeochemical cycle of Fe. However, it is often compounded with other biotic and abiotic processes.

Stable Fe isotopes have provided a potential way to distinguish different biotic and abiotic processes (Dauphas et al. 2017; Johnson et al. 2004). Iron has four naturally occurring stable isotope: ^{54}Fe (5.845%), ^{56}Fe (91.754%), ^{57}Fe (2.119%), ^{58}Fe (0.292%), varying over 6‰ in $^{56}Fe/^{54}Fe$ for the typical natural systems (Dauphas et al. 2017; Meija et al. 2016). Both biotic and abiotic processes could fractionate Fe isotopes, causing significant mass dependent isotope fractionation and leaving unique Fe isotope signatures on the resulting Fe pools (Beard et al. 2003; Dauphas et al. 2017; Johnson et al. 2004). Recently, Amor et al. (2016) observed small but significant mass independent fractionation of the odd ^{57}Fe during magnetite biomineralization by magnetotactic bacteria.

As summarized in Johnson et al. (2004), microorganisms may process Fe in three pathways: 1) lithotrophic and phototrophic metabolisms where Fe^{II} is oxidized to Fe^{III}, 2) dissimilatory Fe metabolism where Fe^{III} is reduced to Fe^{II}, and 3) assimilatory Fe metabolism where Fe is transported into biomolecules. Laboratory-controlled experiments have shown that significant Fe isotope fractionation has been observed during metabolic reduction of aqueous Fe^{III} or mineral Fe^{III} (Beard et al. 1999; Beard et al. 2003; Crosby et al. 2005; Crosby et al. 2007; Johnson et al. 2005) and oxidation of aqueous Fe^{II} (Balci et al. 2006; Croal et al. 2004) by various bacterial strains. Overall, the Fe^{II} compounds as either the reactants or products are enriched in light Fe isotopes relative to Fe^{III} compounds. Abiotic processes such as dissolution, adsorption, diffusion, reduction and oxidation (Brantley et al. 2004; Icopini et al. 2004; Johnson et al. 2008; Roe et al. 2003; Sio et al. 2013; Welch et al. 2003) also significantly fractionate Fe isotopes between Fe species of different binding environments and redox states. Controversy still exists regarding the importance of various biotic and abiotic Fe isotope fractionation effects during biogeochemical cycle of Fe in the natural system (Amor et al. 2016; Brantley et al. 2001; Brantley et al. 2004; Bullen et al. 2001; Icopini et al. 2004).

At present, it is still challenging to characterize the mechanisms of

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Fe metabolism in the phytoplankton (Botebol et al. 2015; Botebol et al. 2014; Lelandais et al. 2016; Sutak et al. 2012). Homeostasis of Fe is strictly regulated in microorganisms, and both reductive and non-reductive pathways have been proposed for Fe uptake by phytoplankton (Andrews et al. 2003; Kosman 2003; Sutak et al. 2008). Iron isotopes might provide useful insights to constrain the mechanisms by which Fe is taken up by phytoplankton (Mulholland et al. 2015; Swanner et al. 2017). Here, two algae species *Chlorella pyrenoidosa* and *Chlamydomonas reinhardtii* were cultured in a Fe-limiting medium where $FeSO_4$ solution was used as the only Fe source. Both algal species are model unicellular eukaryotes in freshwater system with distinct morphology, and produce oxygen as a by-product of photosynthesis. We measured the Fe isotope compositions of the total bulk Fe of the algae and the Fe assimilated into the algal cells. Our aims are to investigate 1) if the uptake of Fe by algae could cause characteristic Fe isotope fractionation signatures, and 2) if the Fe isotope variation allows us to identify the metabolic pathways of Fe.

2. Experimental designs and analysis

2.1. Materials and reagents

The algae *C. pyrenoidosa* and *C. reinhardtii*, and the SE medium gradients (modified from Bristol's solution) (Bold 1949) used in the culture experiments were obtained from the Institute of Hydrobiology, Chinese Academy of Sciences. The SE medium has the following components: $NaNO_3$: 250 mg, $CaCl_2$: 25 mg, $MgSO_4{\cdot}7H_2O$: 75 mg, K_2HPO_4: 75 mg, KH_2PO_4: 175 mg, NaCl: 25 mg, soil extract: 40 ml, $FeCl_3{\cdot}6H_2O$: 0.5 mg, EDTA-Fe (0.901 g of $FeCl_3{\cdot}6H_2O$ dissolved in 10 ml of 1 N HCl, mixed with 10 ml of 0.1 N Na_2EDTA and finally diluted with 980 ml of H_2O): 1 ml, A5 solution (mixture of 2.86 g of H_3BO_3, 1.86 g of $MnCl_2{\cdot}4H_2O$, 0.22 g of $ZnSO_4{\cdot}7H_2O$, 0.39 g of $Na_2MoO_4{\cdot}2H_2O$, 0.08 g of $CuSO_4{\cdot}5H_2O$ and 0.05 g of $Co(NO_3)_2{\cdot}6H_2O$ in 1 L of H_2O): 1 ml, distilled water: 958 ml. In order to prepare Fe-deficiency SE medium used for algal Fe^{II} cultures, Fe-containing components were removed from the SE medium. The culture medium was placed in a reciprocally shaking incubation chamber of 25 ± 1 °C with a fluorescent lamp (86 μmol photons $m^{-2}s^{-1}$) as the light source. A light microscope was used to enumerate the algal cell density. The pH was determined using the Horiba LAQUAtwin pH meter. The fresh, sterile $FeSO_4$ solution (0.01 M) was used as the Fe^{II} source, and was prepared by dissolving $FeSO_4{\cdot}7H_2O$ into deionized water within a glove chamber filled with nitrogen. All the containers, filters and glassware were soaked in HNO_3 solution (pH < 2) for at least 24 h and rinsed three times using 18.2 MΩ Milli-Q deionized water before use. The chemical reagents and acids were all trace metal grades.

2.2. Algal Fe^{II} uptake experiments

Before the Fe^{II} uptake experiments, the algae were acclimated in the Fe-deficiency SE medium for 6 days to harvest the Fe-starved algae used in our culture experiments. We used Fe-starved algae for two reasons: 1) decreasing or excluding the external sources of Fe other than the Fe in the culture medium; 2) speeding up the Fe uptake rate by the algae. As compared to the *C. pyrenoidosa* and *C. reinhardtii* acclimated in SE medium, the Fe-starved counterparts acclimated in Fe-deficiency SE medium exhibited higher Fe^{II} uptake capacities when they were cultured in Fe-deficiency SE medium added with ~9 μM of $FeSO_4{\cdot}7H_2O$ solution (Fig. 1).

The Fe^{II} uptake experiments were conducted with the Fe-starved *C. pyrenoidosa* and *C. reinhardtii* under two scenarios: varying initial Fe^{II} concentrations (concentration-gradient experiments) and varying incubation times (time-course experiments).

2.2.1. Concentration-gradient experiments

A buffering medium (pH = 6) composed of K_2HPO_4 (75 mg/L), NaH_2PO_4 (175 mg/L), $NaNO_3$ (250 mg/L) and NaCl solutions (25 mg/L) was used to culture the algae. This medium in different flasks was spiked with $FeSO_4{\cdot}7H_2O$ solutions of different concentrations (1000 μM, 500 μM, 100 μM, 50 μM, 10 μM, 5 μM). The algae were cultured in each medium under a fluorescent light, and were harvested after 4 h. The total volume of each medium was 100–200 ml. At the end of each culture experiment, the medium was divided into two portions by volume, and both portions were filtered through 0.45 μm cellulose acetate filters under aseptic conditions. The filtered algae in one portion were stored for later determination of Fe concentration and isotope composition. The filtered algae in another portion were mixed with 10^{-2} M EDTA solution. It was found that 4 min of mixing time could elute the maximum amounts of Fe from the algae (data not shown). The algae after EDTA elution were also stored for later analysis. Abiotic control experiments were set up using the same medium but were not cultured with algae.

2.2.2. Time-course experiments

The algae were cultured in the Fe-deficiency SE medium (added with ~60 μM $FeSO_4$ solutions and illuminated 16 h per day by the fluorescent light) for different days: 5 days, 10 days, 15 days, and 20 days for *C. pyrenoidosa*; 3 days, 6 days, 9 days, and 12 days for *C. reinhardtii*. The total volume of each medium was 60–200 ml. At the end of each culture experiment, the algae was processed as before. Similarly, abiotic control experiments were also set up.

The cell density of algae showed a significant increase with the increasing culture days, with slightly faster growth rate for *C. reinhardtii* (0.50×10^4 cell $ml^{-1}day^{-1}$) than *C. pyrenoidosa* (0.31×10^4 cell $ml^{-1}day^{-1}$) (Fig. 2). In addition, the pH values of Fe-deficiency SE medium slightly increased for *C. pyrenoidosa* (Fig. 3A) and significantly increased for *C. reinhardtii* (Fig. 3B). This difference is likely caused by the faster propagation rate of *C. reinhardtii* than *C. pyrenoidosa* (Fig. 2). It is noticeable that the Fe-deficiency SE medium had a more stable starting pH value and more moderate pH variation range as compared to the SE medium. Thus, only algae incubated in the Fe-deficiency SE medium were measured for Fe concentrations and Fe isotope compositions.

2.3. Iron concentration and isotope ratio measurement

The filtered algal samples were repeatedly digested using aqua regia plus HF and $HClO_4$ in acid-cleaned Teflon beakers (7 ml, Savillex) according to the previous method (Song et al. 2011). Finally, HCl was added to eliminate HNO_3 and HF before taking to dryness and re-dissolving in 1 ml 6 N HCl + 0.001% H_2O_2 for chemical purification. Aliquots of the sample digests were measured for Fe concentrations by inductively coupled plasma-optical emission spectrometry (Varian Vista MPX).

A polypropylene chromatographic column (Bio−Rad, diameter: 6.8 mm, height 4.3 cm) filled with AG MP-1 resin (Bio−Rad, 100–200 mesh, chloride form) was used for chemical purification of Fe (Liu et al. 2013; Maréchal et al. 1999; Song et al. 2011). The cleaning and preconditioning protocols of the resin, and matrix and Fe elution procedures can be found in Song et al. (2011). The eluted Fe in 25 ml Teflon beaker was evaporated to dryness at 80 °C after adding one drop of concentrated H_2O_2. To eliminate the chloride ions, the purified Fe was re-dissolved three times with 0.1 ml HNO_3 and evaporated to dryness. Finally, 1% HNO_3 was added to the sample beaker before isotope analysis. An aliquot of purified Fe solution was also measured for Fe concentration. The recovery of Fe by chemical purification is typically above 95% by comparing Fe amounts before and after purification. The procedural control blanks processed along with the samples contain < 0.4% of the total Fe of the samples.

The purified Fe solutions were adjusted by 1% HNO_3 to a concentration of ~5 μg/g before isotope measurement. A high-precision (HR) multi-collector inductively coupled plasma mass spectrometry