Experimental determination of Fe isotope effect for reduction from Fe(III) to Fe(II)

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Project
» Experimental constraints on marine Fe isotope effects - Biology, ligands, and particles (Fe isotope effects)

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Abstract
This dataset includes results from laboratory experiments. Specifically, we report the fractionation of iron (Fe) isotopes (δ56Fe) during the reduction of Fe(III) bound to EDTA, to Fe(II) which binds to ferrozine. Experiments were performed under a wide range of conditions, including various temperatures and using various reductants.

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Dataset Description

All of the experiments described here begin with Fe(III) bound to the organic ligand ethylenediaminetetraacetic acid (EDTA), and proceed as Fe is reduced to Fe(II). The Fe(II)-specific ligand ferrozine (FZ) is added to the experimental solution in order to capture Fe(II) produced during the experiment, similar to methods previously used to capture Fe(II) produced during biological reduction of Fe(III) (Shaked et al., 2004). Fe(II) in the presence of ferrozine forms an Fe(II)FZ3 complex. This complex has a strong purple color, which made it possible to determine reaction extent visually and by absorbance spectrophotometry. The Fe(II)FZ3 complex is also hydrophobic, allowing us to separate it from the solution by passing the solution through a C18 resin (Waters Sep-Pak C18 Plus Light) with a peristaltic pump. The column was then rinsed with 5 mL of seawater and Fe(II)FZ3 was eluted with 5 mL methanol.

Methods & Sampling

2.2 Stability of the Fe-ligand complexes
In order to ensure that our experiments are only capturing the isotope effect of Fe(III) reduction to Fe(II) due to our added reductants, we first assessed whether there was any spontaneous reduction of Fe(III)EDTA, spontaneous oxidation of Fe(II)FZ3, or isotope exchange between these complexes. This was tested by separately preparing concentrated solutions of Fe(II)-FZ3 from natural iron, and Fe(III)double-spike-EDTA from an Fe spike consisting of roughly equal portions 57Fe and 58Fe. The stocks were then diluted into surface seawater collected near Bermuda to final concentrations of 2·10-4 M Fe(III) double-spike, 2·10-4 M Fe(II), 10-3 M FZ, and 10-3 M EDTA. Eight such solutions were prepared, to be subsampled over the next 27 h.
At each timepoint, the Fe(II) was first separated from the experimental solution onto the C18 column and eluted with methanol. Then, Fe(III)-EDTA which remained in the experimental solution was reduced to Fe(II)-FZ3 by the addition of 1.5 mL of 1.4 M hydroxylamine hydrochloride, and separated by adsorption as Fe(II)-FZ3 onto C18 resin. Both the Fe(II) and Fe(III) fractions were then purified for isotope analysis as described below.

2.3 Chemical reduction experiments
10 mL experimental solutions were prepared by adding reagents to a 15 mL polypropylene centrifuge tube in the following order: 1) 100 µL Fe(III), 2) 100 µL EDTA, mixed and allowed to equilibrate for > 2 min to allow EDTA to bind the Fe(III), 3) 1 mL buffer, 4) 100 uL ferrozine, 5) 8.6 mL filtered trace-metal clean seawater collected from the surface ocean near Bermuda, 6) 100 µL reductant added in order to initiate the experiment. Experiments at 60°C and 100°C were performed by immersing samples in a heated water bath before adding reductant, while samples performed at 2°C were immersed in an ice bath.
The progress of reaction was monitored by visually examining tubes for the appearance of purple-colored Fe(II)FZ3. For comparison, a standard was made containing 10 µM Fe(II)-FZ3, equivalent to reducing 5% of the initial Fe(III)-EDTA, so that when the color in the experimental tube matched that of the standard, experiments could be terminated at a reaction extent of about 3-7% completion. The exact extent of reaction was then determined by comparing sample adsorption at 562 nm to iron standards prepared using the ferrozine method (Viollier et al., 2000).

2.4 Photochemical reduction experiments
Photochemical reduction experiments were performed as above, except that no chemical reductant was added. Instead samples were either amended either with glucaric acid or not, and the experiment was initiated by bringing samples outside and exposing them to natural sunlight on a nearly-cloudless summer mid-day in South Carolina.

2.5 Isotope analysis
Methanol samples containing Fe(II)FZ3 were evaporated, then reacted overnight on a hot-plate with 500 µL 0.1N HNO3 and 500 µL concentrated H2O2 to destroy the FZ. Samples were then evaporated again and observed to make sure that no purple color (Fe(II)FZ3) remained. If necessary, samples were reacted again with HNO3 and H2O2 until no purple color remained. Samples were analyzed for Fe stable isotope ratios (δ56Fe) at the University of South Carolina according to published methods (Conway et al., 2013) as modified for analysis of marine particles (Revels et al., 2015). Briefly, samples were amended with a ~1:1 57Fe:58Fe double spike, purified by anion exchange chromatography, and analyzed on a Neptune multi-collector ICP-MS. Sample δ56Fe was analyzed compared to IRMM-014 and the δ56Fe of the FeCl3 used to prepare stock solutions was also analyzed, so that all data are reported here as Δ56Fe, calculated as δ56FeFe(II)-δ56FeFe(III).

Data Processing Description

BCO-DMO Processing Notes:
- added conventional header with dataset name, PI name, version date
- modified parameter names to conform with BCO-DMO naming conventions

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Parameters

Parameter	Description	Units
Sample_ID	The internal sample identification number	unitless
Buffer	The name of the buffer used	unitless
pH	The pH of the reaction solution	unitless
Reductant	The name of the reductant used	unitless
Reductant_conc	The concentration of the reductant	millimolar
Temp	Reaction temperature	degrees Celsius
Replicate	Number of replicate (1; 2; or 3)	unitless
d56Fe	Fe isotope ratio compared to IRMM-014	permil
err	Analytical error in d56Fe	permil
Dd56Fe	Fe isotope ratio compared to the Fe starting material	permil

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Project Information

Experimental constraints on marine Fe isotope effects - Biology, ligands, and particles (Fe isotope effects)

Coverage: Eastern Tropical Pacific

Extracted from the NSF award abstract:

Iron is an essential micronutrient for marine phytoplankton, which plays a key role in the global carbon cycle and marine ecosystems, and there is a need to better understand the sources and sinks of this essential micronutrient in the oceans. Iron isotopes are a recently developed tracer of iron biogeochemical cycling, and new iron isotope results indicate that iron-binding ligands may play a critical role in the isotopic fractionation of dissolved iron. At present, however, the quantitative importance of iron dissolution or biological iron uptake in the isotopic fractionation of dissolved iron in the oceans is not well-known. In this study, researchers from the University of South Carolina and the Bermuda Institute of Ocean Sciences will collect and analyze samples from the Bermuda Atlantic Time-series Station (BATS) in order to; 1) measure the rate and isotope fractionation of iron dissolution from natural aerosols and oxic sediments in the presence of natural iron-binding ligands, and; 2) examine how phytoplankton fractionate iron isotopes during biological uptake. Results from this study will advance the development of iron isotopes as a tool for tracing iron biogeochemical cycling in the oceans.

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