

PHOTOSYNTHESIS AND BIOGENIC FRACTIONATION OF MAGNESIUM ISOTOPES: AN EXPERIMENTAL STUDY. J. R. Black^{1,2}, Q.-Z. Yin² and W. H. Casey^{1,2}, ¹Department of Chemistry, ²Department of Geology, University of California, Davis, CA, 95616. (jrblack@ucdavis.edu, yin@geology.ucdavis.edu, whcasey@ucdavis.edu).

Introduction: The advent of multicollector inductively-coupled-plasma mass-spectrometry has opened the door for the precise measurement of “non-traditional” stable isotopes of alkali earth and transition metals [1]. These metals play an important role in biological systems as micro- and macro-nutrients for plants and microorganisms. As a result, the biogenic use of these metals leads to unique geochemical signatures in the environment. Recent interest has been focused on environmental distribution of iron isotopes during their dissimilatory redox cycling by microorganisms [2]. There is still much debate over the relative influence of biotic vs. abiotic processes as a cause for these observed fractionations [3].

One alkali earth metal important to all photosynthetic life on the planet is magnesium. Magnesium is at the center of the chlorophyll molecule, and therefore, central to the primary oxygen evolving process on the planet. Magnesium has three stable isotopes in relatively large natural abundance ($^{24}\text{Mg} = 78.992\%$, $^{25}\text{Mg} = 10.003\%$ and $^{26}\text{Mg} = 11.005\%$), making magnesium an ideal element for studying whether there is any evidence for the biogenic fractionation of its isotopes. Bioavailable magnesium has only one redox state (Mg^{2+}), which potentially makes it easier to interpret an observed fractionation of the isotopes.

Experimental: A series of laboratory experiments were designed to test directly whether or not microorganisms and plants fractionate magnesium isotopes during their growth cycle (see Results/Discussion). The collected Mg-bearing biological samples were digested in 3ml of conc. HNO_3 in teflon cups sealed inside high pressure stainless-steel bombs (Parr acid-digestion bomb) to break down any organic matter. The magnesium was then purified on columns of cation exchange resin [4]. A yield of $100 \pm 5\%$ magnesium was obtained from the columns and the samples were then made up in 0.1N HCl to $\sim 400\text{ppb}$. Isotopic ratios ($^{26}\text{Mg}/^{24}\text{Mg}$, $^{25}\text{Mg}/^{24}\text{Mg}$) were measured using a standard-sample-bracketing technique on an MC-ICP-MS (Nu Instruments Ltd.) at the University of California, Davis relative to the international standards DSM-3 and Cambridge-1.

Results/Discussion: In a recent study [4] we have shown that the magnesium in chlorophyll-a harvested from a cyanobacterium (*Synechococcus elongatus*) is slightly depleted in the heavy isotopes of magnesium relative to the culture medium in which the cyanobac-

teria were grown. Figure 1 shows these results relative to the international standards DSM-3 and Cambridge-1. The result is consistent with a general light isotope enrichment in biological systems, such as that observed in the carbon isotope record of marine phytoplankton biomass relative to the dissolved CO_2 source [5].

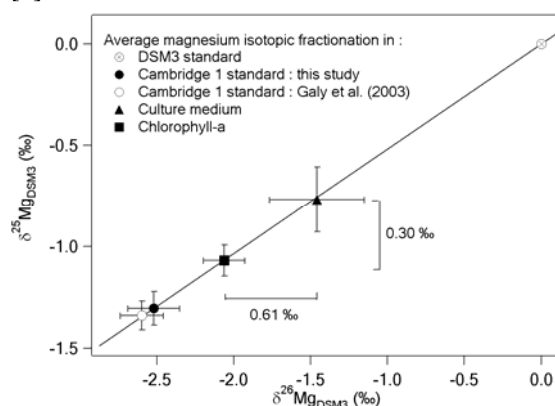


Figure 1: Magnesium-isotope ratios in samples of chlorophyll-a and culture medium relative to DSM3 standard $\pm 2\sigma$.

This fractionation was postulated as potentially occurring during the biosynthesis of chlorophyll-a in the cyanobacteria's life cycle, as the isotopic composition of the culture medium does not change over time. Magnesium-isotope fractionation may occur during the insertion of magnesium into protoporphyrin IX (Figure 2), a process catalysed by the magnesium-chelatase enzyme [6]. Similar chelating resins, such as the Merrifield peptide resin, can cause isotopic fractionation in purely inorganic systems [7]. Further studies are underway to determine whether the observed fractionation can be seen in the cytoplasm of the bacteria, which may suggest other mechanisms such as uptake and transport of magnesium across cell membranes causing the observed fractionation.

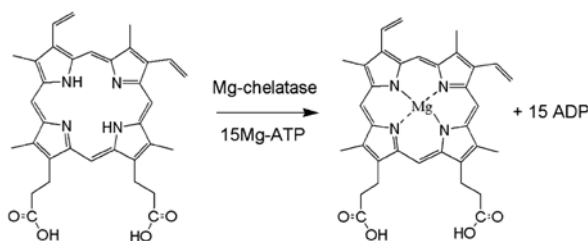


Figure 2: Insertion of Mg into protoporphyrin IX molecule.

To test the hypothesis that magnesium may be fractionated by the uptake and transport mechanisms of an organism, a series of experiments using hydroponically grown wheat are underway. Wheat seedlings were germinated and grown in 18-L solutions of 0.3-0.5mM Mg. The nutrient solution was regularly topped back up to 18 L as the plants grew and the degree of magnesium depletion monitored by taking samples at regular intervals of the plant's growth cycle and measuring the [Mg] by atomic absorption spectroscopy (Figure 3).

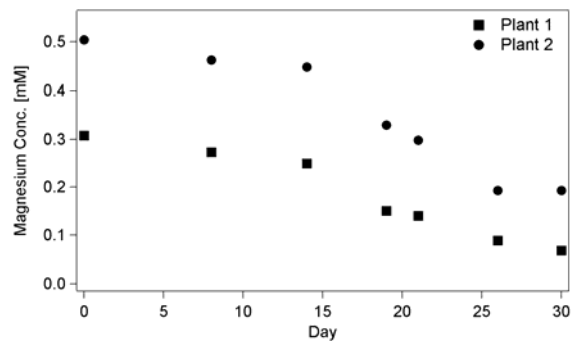


Figure 3: Magnesium depletion in the nutrient solution of wheat plants grown over a period of 30 days.

Figure 3 shows that the wheat plants used ~ 60 to 80% of the magnesium available to them over the growth period of 30 days. Preliminary results from measuring the isotopic composition of a wheat nutrient solution show an interesting result. The nutrient solutions end up slightly depleted in heavy isotopes ($\Delta^{26}\text{Mg} = -0.42\text{‰}$; $\Delta^{25}\text{Mg} = -0.20\text{‰}$, relative to the original composition) after 30 days of growth (Figure 4). Although within the measured error, the depletion of heavy isotopes in the growth medium after 19 days of growth appears to be larger ($\Delta^{26}\text{Mg} = -0.70\text{‰}$; $\Delta^{25}\text{Mg} = -0.34\text{‰}$, relative to the original composition).

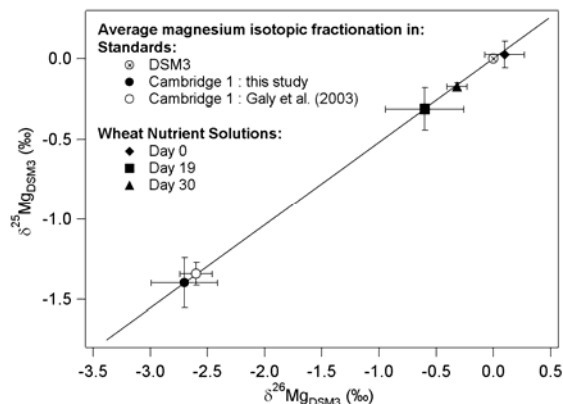


Figure 4: Magnesium-isotope ratios in samples of wheat nutrient solution relative to DSM3 standard $\pm 2\sigma$.

This result seems counterintuitive if the fractionation of isotopes were to follow a Rayleigh distillation.

It is possible and quite likely that the plants uptake/outlet mechanisms for utilizing magnesium change with time or with the concentration of nutrients in the growth solution. One thing is clear from these preliminary data: Mg isotope fractionation through membrane transport prefers heavy isotopes, leaving the resulting solution isotopically lighter. Similar effects have been observed in a study of Fe isotopes through cell membranes [8]. If this observation stands up to further tests in both plants and microorganisms, we could rule out transport mechanisms being responsible for the observed light isotope enrichment during the photosynthesis of chlorophyll-a in cyanobacteria [4].

Conclusions: Evidence for the biogenic fractionation of magnesium isotopes has clearly been shown [4]. However, the mechanisms governing this fractionation and whether it will be organism specific have yet to be proven. Preliminary results from experiments growing wheat plants suggests that they preferentially take up the heavy isotopes of magnesium. This result would appear to contradict that of our initial studies using cyanobacteria [4] where the magnesium composition of chlorophyll-a was found to be isotopically light relative to its origin. This may reflect a difference between the way microorganisms and higher plants utilise magnesium. We are conducting experiments to identify what effect magnesium uptake mechanisms, in bacteria and higher plants, have upon the isotopic composition of the intracellular reservoir of magnesium.

Magnesium isotopic fractionations associated with chlorophyll-a may provide a new tool for identifying the abundance of molecular oxygen producing photosynthesis in specific environments. If isotopic signatures are characteristic of specific enzymatic processes and are incorporated into minerals such as carbonates, we may be able to trace the most important autotrophic process through the rock record. The biogenic fractionation of "non-traditional" stable isotopes may become an important tool for identifying where and how life evolved here on our planet, and for potentially identifying a record of life on other planets such as Mars.

References: [1] Johnson C.M., Beard B.L. and Albarède F. eds. (2004), *Rev. Min. & Geochem.*, 55. [2] Anbar et al. (2006), *AGU Fall Meeting*, Abstract #V14C-05. [3] Johnson, C. M. and Beard, B. L., *Science*, 309, 1025-1027. [4] Black, J. R. et al. (2006), *Geo. Cosmo. Acta*, 70, 4072-4079. [5] Valley, J. W. and Cole, D. R., eds., (2001), *Rev. Min. & Geochem.*, 43, [6] Willows, R. D. (2003), *Nat. Prod. Rep.*, 20, 327-341. [7] Kim, D. W. et al., (2003), *J. Coll. & Inter. Sci.*, 263, 528-532. [8] Croal et al., (2004), *Geo. Cosmo. Acta*, 68, 1227-1242.