An experimental study of magnesium isotope fractionation in Chlorophyll-a Photosynthesis

ARTICLE *in* GEOCHIMICA ET COSMOCHIMICA ACTA · AUGUST 2006

Impact Factor: 4.33 · DOI: 10.1016/j.gca.2006.06.010

3 AUTHORS, INCLUDING:

Jay R. Black
University of Melbourne
33 PUBLICATIONS **367** CITATIONS

Title:
An experimental study of magnesium-isotope fractionation in chlorophyll-a photosynthesis

Author:
Black, J R
Yin, Q Z, University of California, Davis
Casey, W H

Publication Date:
08-01-2006

Publication Info:
Postprints, UC Davis

Additional Info:
The published version of this article is available at: www.elsevier.com/locate/gca

Abstract:
Measurements are presented of the magnesium isotopic composition of chlorophyll-a, extracted from cyanobacteria, relative to the isotopic composition of the culture medium in which the cyanobacteria were grown. Yields of 50-93% chlorophyll-a were achieved from the pigment extracts of Synechococcus elongatus, a unicellular cyanobacteria. This material was then digested using concentrated nitric acid to extract magnesium. Separation was accomplished using columns of cation-exchange resin, which achieved a 103 +/- 10% yield of magnesium from chlorophyll-a. This procedure ensured accurate measurement of the magnesium-isotopic ratios without isobaric interferences using a multi-collector inductively coupled plasma mass spectrometry (MC-ICP-MS). We find a slight depletion in the heavier isotopes of magnesium in chlorophyll-a relative to culture medium, early growth phase: Delta Mg-26 = -0.71(+/- 0.35)parts per thousand and Delta Mg-25 = -0.37(+/- 0.18)parts per thousand; late growth phase: Delta Mg-26 = -0.53(+/- 0.20)parts per thousand and Delta Mg-25 = -0.26(+/- 0.11)parts per thousand, due to an apparent mass-dependent fractionation. We suggest that the small fractionation results from chelation during intracellular processes. A likely candidate for this chelation step involves the magnesium-chelatase enzyme, which mediates the insertion of magnesium to the tetrapyrrole ring during chlorophyll-a biosynthesis. Proof of this hypothesis can be tested with biological controls whereby steps in the enzymatic pathways of chlorophyll synthesis are selectively suppressed.

Geochim. Cosmochim Acta June 07,2006

An Experimental Study of Magnesium-Isotope Fractionation in Chlorophyll-a Photosynthesis

Jay R. Black

Department of Chemistry

Department of Geology,

University of California, Davis, CA 95616.

jrblack@ucdavis.edu

Qing-zhu Yin

Department of Geology,

University of California, Davis, CA 95616.

qyin@ucdavis.edu

and

William H. Casey*

Department of Chemistry

Department of Geology,

University of California, Davis, CA 95616.

whcasey@ucdavis.edu

keywords: photosynthesis, magnesium isotopes, isotope fractionation, chlorophyll, cyanobacteria, biogeochemistry

running title: Mg-isotope fractionation in chlorophyll-a

Abstract

Measurements are presented of the magnesium isotopic composition of chlorophyll-a, extracted from cyanobacteria, relative to the isotopic composition of the culture medium in which the cyanobacteria were grown. Yields of 50-93% chlorophyll-a were achieved from the pigment extracts of *Synechococcus elongatus*, a unicellular cyanobacteria. This material was then digested using concentrated nitric acid to extract magnesium. Separation was accomplished using columns of cation-exchange resin, which achieved a 103 ± 10% yield of magnesium from chlorophyll-a. This procedure ensured accurate measurement of the magnesium-isotopic ratios using a multi-collector inductively coupled plasma mass spectrometry (MC-ICP-MS) without isobaric interferences. We find a slight depletion in the heavier isotopes of magnesium in chlorophyll-a relative to culture medium, early growth phase: $\Delta^{26}Mg = -0.71(\pm0.35)‰$ and $\Delta^{25}Mg = -0.37(\pm0.18)$ ‰; late growth phase: $\Delta^{26}Mg = -0.53(\pm0.20)‰$ and $\Delta^{25}Mg = -0.26(\pm0.11)$ ‰, due to an apparent mass-dependent fractionation.

We suggest that the small fractionation results from chelation during intracellular processes. A likely candidate for this chelation step involves the magnesium-chelatase enzyme, which mediates the insertion of magnesium to the tetrapyrrole ring during chlorophyll-a biosynthesis. Proof of this hypothesis can be tested with biological controls whereby steps in the enzymatic pathways of chlorophyll synthesis are selectively suppressed.

1. Introduction

Rates of energy conversion by photosynthesis across the planet are enormous and account for ~105 gigatonnes of carbon per year (Field et al., 1998). This primary production is important to all geochemical cycles. Chlorophyll-a is frequently used as a marker of biomass (e.g. Falkowski et al., 1998; Roman et al., 2005) and the global distribution can be monitored by satellite. Magnesium is the metal center in the chlorophyll molecule and thus is central to all photosynthesis, and hence to the history and geochemistry of life on the planet.

In this paper we test the hypothesis that magnesium isotopes fractionate during biosynthesis of chlorophyll-a, one of the most common types. The ubiquity of the naturally occurring isotopes of magnesium, ^{24}Mg (78.7%), ^{25}Mg (10.1%) and ^{26}Mg (11.2%) leads us to the question: 'Is there a fractionation of these isotopes during the synthesis of chlorophyll and could this be used as a potential biomarker?'

The potential geochemical significance of this fractionation is considerable, since the fractionation may be affected by temperature or other environmental conditions and thus the isotopic composition of chlorophyll may provide a record of geochemical conditions. There is some evidence in the literature of isotopic fractionation in both chlorophyll-a and -b (Galy et al., 2001). Young and Galy (2004) report δ^xMg values (part per thousand variations, x=25, 26, see eq. 1) relative to the DSM3 standard of δ^{26}Mg = -1.451‰, δ^{25}Mg = -0.741‰ for spinach chlorophyll-a, and δ^{26}Mg = -2.349‰, δ^{25}Mg = -1.204‰ for spinach chlorophyll-b. However, the magnesium-isotopic composition of the culture medium from which the spinach was grown was not known, as they used chlorophylls from a commercial source for their study. Similarly, Ra et al., (2003) report

a large difference in the δ^{26}Mg of chlorophyll-a and –b from several commercial sources, including cultured planktonic species and marine phytoplankton samples, but did not detail the data in their abstract.

It is reasonable to expect a magnesium-isotope fractionation given our current understanding of the biosynthetic pathways of chlorophyll. Fractionation could take place during the insertion of magnesium into protoporphyrin IX (Figure 1), which involves the magnesium-chelatase enzyme (e.g., Willows, 2003). This enzyme binds the Mg(II) tightly and introduces it into the porphyrin in a late state. Similar chelating resins and magnesium-binding molecules are known to induce an isotopic fractionation in purely inorganic systems (e.g. ^{26}Mg enrichment on functionalized Merrifield peptide resins, Kim and Kang, 2001; Kim et al., 2002, 2003).

Recent studies by Buchachenko (2001), Buchachenko et al. (2005a, 2005b), suggest a wholly different kinetic pathway that may involve coupling of the electron- and nuclear-spins in a free-radical reaction. The essence of this unconventional pathway is that decay of a transient free radical proceeds much faster (e.g. 2-3 times for ATP synthesis, Kuznetsov et al. 2005) in the presence of magnetic ^{25}Mg than diamagnetic ^{24}Mg (Buchachenko et al., 2005a). The difference is entirely kinetic and relates to the relative rates of relaxation of excited electrons in the vicinity of the nuclear spin. If a kinetic advantage is to be gained by concentrating the magnetic isotope, one wonders if the enzymatic pathways concentrate ^{25}Mg. If so, a mass-independent magnesium-isotope fractionation is expected.

2. Experimental Methods

2.1 Growth of Cyanobacterial Cultures

A culture of *Synechococcus elongatus* (*Anacystis nidulans*, PCC 7942), a unicellular cyanobacteria, was provided by Prof. John Meeks at the University of California, Davis. This cyanobacteria was chosen for this study because it produces only chlorophyll-a, thus eliminating the need to separate chlorophyll-a and –b from the pigment extract. Samples of cyanobacteria were grown in a freshly prepared BG11 culture medium (Table 1) by dissolving ~ 1.65 g in 1 L of water (18 MΩ resistance) and adding 1 ml of a trace-metal mix (Table 1). The pH of the culture medium was adjusted to 7.1 with a small amount of 0.1 N HCl and the stock solutions were autoclaved at 121^{o}C for 20 minutes in order to sterilize them. Sample stocks of *S. elongatus* were inoculated in a laminar-flow hood with 1 ml of a stock culture to 100-400 ml of BG11 broth. Samples were grown in an agitating water bath at 25^{o}C ± 0.2 degrees under two full-spectrum fluorescent globes providing a light intensity of 4200 to 5000 foot candles, measured using a spherical Li-COR radiation sensor.

The growth phases of the *S. elongatus* stock cultures were determined turbidimetrically, by measuring the solution absorbance from 700 to 350nm in a 1-cm pyrex cuvette every 2-3 days. Typical results are shown in Figure 2, where we combine absorbance measurements at 750nm from two stock solutions in two growth phases over 55 days.

2.2 Pigment Extraction and Purification on DEAE-Sepharose

A series of ten cyanobacterial cultures were grown for extraction of the pigments, including chlorophyll-a, from different stages of the cultures growth (Figure 2). Samples

from a culture were first filtered on a Whatman GF/F glass microfiber filter. The separated cyanobacteria were rinsed thoroughly with water to eliminate any residual BG11 broth. Pigments were then extracted from the washed cyanobacteria by soaking the glass microfiber filter twice in 10 ml of methanol with 5 minutes of sonication. The methanol extracts were combined and then filtered through a glass frit. In all of these treatments, the filtrate was protected from light to prevent photodegradation of the chlorophyll-a. This extraction cleanly separates the green chlorophyll pigments from other pigments, such as the biliproteins (e.g. phycocyanin, a blue copper-bound protein). In order to determine the yield of the extraction, we measured the UV-Vis spectrum of each chlorophyll solution over the range 750-330 nm in 1 cm pyrex cuvettes (see Figure 3). After this step, the solutions were concentrated by rotary evaporation to dryness. The dried pigment was then dissolved in 3-5ml of acetone.

Purification of the chlorophyll from the acetone extract was carried out on a jacketed column (4^{o}C) of DEAE-Sepharose with a 1-cm I.D. and 2-cm bed height according to the methods of Omata and Murata (1980). Chlorophyll-a is weakly bound to diethylaminoethyl groups on the resin while other major pigments (e.g., zeaxanthin and β, β-carotene) elute cleanly with fresh acetone. The eluent was then changed to a mixture of 77% acetone and 23% methanol, which elutes the chlorophyll-a from the column. In Figure 3 we show the UV-Vis spectrum of the fractions collected off the column. The pure chlorophyll-a fraction was evaporated to dryness under vacuum and taken up in methanol to determine the yield.

The yield was determined using UV-Vis spectra (Figure 3), by averaging concentration estimates from the absorbance maximum of 665.5 nm and from the

shoulder at 652 nm. There are no interferences from other pigments at these wavelengths. Extinction coefficients for chlorophyll-a in methanol (71.43 l $mmol^{-1}$ cm^{-1} at 665.2nm and 31.65 l $mmol^{-1}$ cm^{-1} at 652 nm in methanol; Porra et al., 1989) were used. We estimated the extinction coefficients in a 77% acetone, 23% methanol mixture by diluting an acetone extract with methanol. The amounts of extracted chlorophyll and yields are reported in Table 2. The biggest loss of chlorophyll is in the rotary evaporator, where solution bumps into the solvent trap. These losses do not cause magnesium-isotope fractionation (see below). Losses due to photodegradation or magnesium leaching from the chlorophyll tetrapyrrole ring are a few percent at most.

Magnesium bound to chlorophyll is not the largest reservoir of Mg(II) in the cyanobacterium as that there is a large concentration in the cytoplasm (Jasper and Silver, 1977). Therefore, in order to avoid contamination of the samples, this intracellular fluid was separated cleanly from the chlorophyll extract by filtration and anion-exchange treatment. Retention of the extracted chlorophyll-a, with a central coordinated magnesium, on an anion-exchange column allows separation from the various other organic pigments. In this manner, we separate the non-chlorophyll magnesium from the chlorophyll-bound magnesium. The only source of magnesium in the sample is therefore from the extracted chlorophyll.

2.3 Magnesium Purification on AG 50W-X12 Cation-Exchange Resin

Magnesium was liberated from the porphyrin ring by acid digestion in 3 ml of concentrated, and ultrapure, nitric acid. The acid digestions were undertaken in teflon cups that were sealed in a stainless-steel bomb (Parr acid-digestion bomb), which was placed in an oven at 150^{o}C for 4 hours. To further eliminate chemical interferences in the

sample, the aqueous magnesium solution was passed through a cation-exchange column using a AG 50W-X12 200-400 mesh resin (Bio-Rad). The resin was prepared according to the methods of Chang et al., (2003) and the glass columns had a 5 mm I.D. and 7.5 cm bed height. The resin was cleaned with 6 ml of 6M HNO_3 between samples, followed by a 4 ml rinse with water. In a final step, the columns were charged with 1 M HNO_3.

The columns were calibrated to identify the elution peak of the magnesium-only fraction (Figure 4) using both the BG11 broth and a stock solution of magnesium sulphate (the same magnesium salt used in BG11 mixture, Table 1) in 1 M HNO_3, which had been treated identically as the samples. The first milliliter of sample was fully loaded to the column, followed by a 3 ml rinse. The level of eluent above the resin bed was kept between 3-4 ml and the columns allowed to flow under gravity. This method cleanly separates Mg(II) from other metals, as determined by repeated calibrations (Figure 4), where metal concentrations were determined by Atomic Absorption Spectrometry and/or by ICP-MS. A full 103 ±10 % yield (magnesium recovery) from the cation-exchange column is achieved (see Table 2). It is essential to ensure full recovery of magnesium from the column in order to avoid significant magnesium isotopic fractionation induced by cation-exchange resin (Chang et al., 2003). After collection of the pure magnesium fraction in Teflon beakers, these fractions were evaporated at 80^{o}C. Aliquots of the samples were then taken and diluted with 0.1 M HCl so that final concentration of magnesium was around 400 ppb.

2.4 Measurement of the Isotopic Composition of Magnesium

Magnesium isotopic ratios were measured using a Nu instruments multi-collector inductively coupled plasma mass spectrometer (MC-ICP-MS). We use 0.1 M HCl as a matrix for standards and samples to avoid potential $^{12}C^{14}N$ interference on ^{26}Mg observed when dilute HNO_3 was used. Two international standards, DSM3 and Cambridge 1, were chosen as stable reference materials. We used the standard-sample bracketing technique to monitor drift and instrumental mass fractionation. Both standards were measured repeatedly between samples to monitor the established isotopic differences between DSM 3 and Cambridge 1 (Galy et al., 2003). The isotopic ratio of the DSM3 standard was then extrapolated to the time of sample measurement in order to standardize the data (see results section). It is essential to prepare standards and samples from the same acid stock solutions at the same time immediately before mass spectrometric analyses. Negligible cross-contamination of samples and standards was achieved using three separate 0.1 M HCl rinses of increasing purity before sample analysis. The third acid is used for Mg background subtraction. The use of a DSN-100 desolvating nebuliser also helped to reduce interferences and boost the signal intensity to 6-9 volts for ^{24}Mg on a 10^{11} Ω resistor faraday cup.

3. Results

The ratios of $^{26}Mg/^{24}Mg$ and $^{25}Mg/^{24}Mg$ measured using the MC-ICP-MS were standardized against the international standard DSM3 by converting these values to a $\delta^{x}Mg$:

$$\delta^{x}Mg = \left(\frac{(^{x}Mg/^{24}Mg)_{Sample}}{(^{x}Mg/^{24}Mg)_{DSM3}} - 1 \right) \times 1000 \qquad (1)$$

where x=26, or 25.

The results, and a comparison with other literature studies, are summarized in Table 3, which reports the average $\delta^{x}Mg_{DSM3}$ value from a number of replicate measurements of the samples and standards. For DSM3, the $\delta^{x}Mg_{DSM3}$ value is 0 by definition. Table 3 also reports the per mil difference between the chlorophyll-a samples and the culture medium, which we define as $\Delta^{x}Mg$, where x= 25, 26. The uncertainty is reported at 95% confidence level (2σ).

A plot of $\delta^{25}Mg_{DSM3}$ vs $\delta^{26}Mg_{DSM3}$ (Figure 5) shows that magnesium in chlorophyll-a is slightly depleted in the heavier isotopes (early growth phase : $\Delta^{26}Mg$ = -0.71(±0.35)‰ and $\Delta^{25}Mg$ = -0.37(±0.18) ‰; late growth phase : $\Delta^{26}Mg$ = -0.53(±0.20)‰ and $\Delta^{25}Mg$ = -0.26(±0.11) ‰) relative to the culture medium. Note that the errors include uncertainties for magnesium isotopes in both chlorophyll-a as well as in the culture medium. These fractionations of magnesium isotopes in chlorophyll-a are small but observed in all experiments and are clearly resolved from the magnesium-isotopic composition of the culture medium (see Table 3 and Figure 5). Two blank samples were run through the entire experimental method in order to quantify the amount of magnesium in a blank. Relative to the extracted magnesium from chlorophyll-a, where an average yield of 103 ± 10% was obtained (Table 2), the background magnesium in the blanks was 4.7 ± 1.7%. The average isotopic composition for the $MgSO_4$ stock is close to that of the culture medium before growth, as one would expect if there were no fractionation induced by the extraction procedure or column chemistry. The isotopic composition of the culture medium after growth is also, on average, very close to that of culture medium before growth and to the $MgSO_4$ stock, suggesting that magnesium

bound to chlorophyll-a is a small fraction of the total, which is typical. Therefore, an overall average for isotopic composition of the culture medium, which combines the results of the $MgSO_4$ stock and the culture medium before and after growth, is reported in Table 3 and plotted in Figure 5b. The resulting uncertainty appears somewhat large, but is the most conservative estimate for the magnesium-isotopic composition of the starting materials.

It is particularly important to note that we employ an overall range of the isotopic compositions for magnesium extracted from chlorophyll-a (e.g. "early growth phase" pools together six samples) as a conservative estimate of the uncertainty (±0.285) rather than the error on individual measurements (e.g. ±0.034 for C023). Therefore our uncertainty includes isotopic variations from yield variations, if such an effect is present. However, we find no correlation between the yield of extracted chlorophyll-a (Table 2, column 4) and the $\delta^{25}Mg_{DSM3}$ or $\delta^{26}Mg_{DSM3}$ observed in the individual samples (Table 3), thus demonstrating that the process of extracting the chlorophyll did not produce the observed fractionation. The subsequent magnesium extraction from this chlorophyll-a is 103 ± 10% (Table 2, column 5), and again there is no correlation between the Mg yield in individual samples and their magnesium-isotopic fractionation indicating that there is no fractionation induced by the cation-exchange column chemistry.

The precision for repeated measurements of the Cambridge standard during the course of this study (2σ for $\delta^{26}Mg = \pm 0.17$) is comparable to that of Galy et al., 2003 (± 0.14). The similarity in uncertainty between repeated measurement of samples and chemically pure standards (see Fig. 5a) indicates that the associated uncertainty is not due to chemical processing of the samples and the so-called matrix effect. We report the

overall variation among different experimental sessions, rather than individual measurement errors, as our experimental uncertainty, because we believe this practice provides the most conservative estimate of errors (Fig. 5b).

4. Discussion

The simplest interpretation of these experiments is that the fractionation of magnesium isotopes occurs in growth of the cyanobacteria and synthesis of chlorophyll-a. Our results show a depletion in the heavier isotopes of magnesium, early growth phase: $\Delta^{26}Mg = -0.71(\pm0.35)‰$ and $\Delta^{25}Mg = -0.37(\pm0.18)$ ‰; late growth phase: $\Delta^{26}Mg = -0.53(\pm0.20)‰$ and $\Delta^{25}Mg = -0.26(\pm0.11)$ ‰), relative to the culture medium (Figure 5).

The step that causes fractionation in chlorophyll synthesis is unknown, but we suspect that it is related to a chelation step, since it is well documented that chelation can cause fractionation of magnesium isotopes on resins (Chang et al., 2003; Kim et al., 2002; 2003; Kim and Kang, 2001). The precise mechanism for insertion of magnesium into the tetrapyrrole ring (protoporphyrin IX, Figure 1) during the biosynthesis of chlorophyll is not known. However, it has been identified that the enzyme magnesium chelatase enhances the rate of magnesium insertion into protoporphyrin IX through a protein backbone of three subunits (ChlI, ChlD, ChlH) and the hydrolysis of adenosine triphosphate (ATP) (Walker and Willows, 1997). The BchI/ChlI subunit has the highest ATPase activity and enhances the rate of exchange of phosphate between ATP and adenosine diphosphate (ADP) (Hansson and Kannangara, 1997). The BchH/ChlH subunit also has a high ATPase activity (Hansson and Kannangara, 1997) and binds the proptoporphyrin IX, and possibly the magnesium, in a similar manner to cobaltochelatase

enzymes (Willows, 2003). Walker and Willows (1997) propose that the hydrolysis of ATP is so critical to the function of magnesium-chelatase, as it allows for the labilisation of a water molecule from the coordination sphere of magnesium before insertion to protoporphyrin IX. They make this postulation based upon a correlation between the rate of insertion of magnesium to a porphyrin and the rate of water labilisation from the magnesium coordination sphere in relation to other divalent metal ions:

Cu > Zn > Mn, Co, Fe > Ni > Cd >> Mg

It is in the steps where magnesium is bound to the protein backbone of magnesium chelatase that a fractionation of the isotopes may occur. Without understanding the exact mechanism of magnesium insertion, it can be speculated that the functional groups binding magnesium before insertion preferentially bind the lighter isotopes. This pathway would account for the depletion in the heavier isotopes.

Chlorophyll-a is contained within the thylakoids, which in the case of *S. elongatus* form photosynthestic lamellae (Pl, Figure 6) within the cytoplasm of the cell. Therefore, the magnesium reservoir used in chlorophyll-a biosynthesis may come from the cytoplasm. *Synechoccocus elongatus* has an approximate cell volume of $2x10^{-12}$ cm^3 (Figure 6), and the magnesium in extracted chlorophyll represents ~0.1 to 0.6% of the total magnesium reservoir in the culture medium, as determined by atomic absorption spectroscopy. The transport and regulation of metals across the cell walls, membranes and in intracellular processes is controlled by diffusion, group translocation and active transport (Jasper and Silver, 1977). Studies have shown that there is a large excess of magnesium in the bacterial cells, exceeding culture medium concentrations (Jasper and Silver, 1977); and therefore, the reservoir of magnesium in the cells and broth is always

much larger than that consumed by the production of chlorophyll-a. If the transport mechanisms which concentrate magnesium in the cytoplasm of cells were responsible for the observed isotopic fractionation in chlorophyll-a, then we would expect to see a slight concentration of the heavy isotopes in the culture medium after growth. If anything, the results show a slight depletion in the heavy isotopes in the culture medium after growth (although well within analytical uncertainty), so it is unlikely that transport of magnesium across the cell wall and membranes is the source of the observed fractionation in chlorophyll-a. This hypothesis could be tested using biological controls that would affect the uptake of magnesium, such as varying the concentration of cobalt and manganese in the culture medium as these metals affect the accumulation of magnesium in bacteria (Jasper and Silver, 1977).

Other prokaryotic species, such as *Salmonella* and *E. coli*, have been used to investigate intracellular transport mechanisms (see review in Silver and Walderhaug, 1992). These studies have been used to develop an intricate understanding of ion transport mechanisms within bacteria, which are governed by various enzymes. The activity of these enzymes in turn is affected by the extracellular and intracellular reservoir of magnesium, and other environmental variables, such as temperature and light intensity. The concentration of chlorophyll-a within *S. elongatus* is known to be inversely related to the light intensity during growth and directly related to temperature (Allen, 1968). Further experiments are required to test whether these physical-environmental properties have any effect on the observed magnesium isotopic fractionation, and are currently underway, and a direct measure of cytoplasm isotopic compositions.

5. Conclusions

The magnesium-isotopic values that we report here for chlorophyll-a, in an early growth phase, are similar to those reported by Young and Galy (2004), all relative to the DSM3 standard. The isotopic fractionation between the culture media and magnesium extracted from chlorophyll-a is shown for the first time to be slightly depleted in the heavier isotopes (early growth phase : $\Delta^{26}Mg = -0.71(\pm0.35)‰$ and $\Delta^{25}Mg = -0.37(\pm0.18)$ ‰; late growth phase : $\Delta^{26}Mg = -0.53(\pm0.20)‰$ and $\Delta^{25}Mg = -0.26(\pm0.11)$ ‰). We suggest that the depletion of heavier magnesium is due to a mass-dependent, preferential binding of ^{24}Mg in a chelation step, possibly to the protein backbone of magnesium-chelatase, before insertion in protoporphyrin IX. To test this hypothesis requires sets of biological controls beyond those that we include here.

Acknowledgements

We thank Professors David Britt, John Meeks and Albert Galy for providing advice and material for these experiments. We thank Benjamin Jacobsen for his assistance in the MC-ICP-MS lab. The isotopic measurements were made at the UC Davis Interdisciplinary Center for Plasma Mass Spectrometry (UCD-ICP-MS) funded by NSF and UC Davis. This work is contribution No. 0012 from the UCD-ICP-MS. This research was supported by the U.S. DOE Office of Basic Energy Sciences via grant DE-FG03-02ER15325 to WHC and by NASA Cosmochemistry Program (NNG05GGN22G) and Origins of Solar Systems Program (NNG05GN03G) to QZY.

Table 1: Composition of BG11 culture medium and trace metal mix added to broth.

BG11 culture medium	Trace-metal mix
$NaNO_3$ = 75.00 g	H_3BO_3 = 2.8601 g L^{-1}
K_2HPO_4 = 2.0000 g	$MnCl_2.4H_2O$ = 1.8112 g L^{-1}
$MgSO_4$ = 1.8311 g	$ZnSO_4.7H_2O$ = 0.221 g L^{-1}
$CaCl_2.2H_2O$ = 1.8028 g	$Na_2MoO_4.2H_2O$ = 0.3908 g L^{-1}
Citric acid.H_2O = 0.3251 g	$CuSO_4.5H_2O$ = 0.0793 g L^{-1}
Ferric citrate.$6H_2O$ = 0.4152 g	$Co(NO_3)_2.6H_2O$ = 0.0495 g L^{-1}
EDTA = 0.0504 g	
Na_2CO_3 = 1.0011 g	
NH_4Cl = 0.0655 g	

Table 2: The initial amounts and final yields of chlorophyll-a and magnesium from *S. elongatus* after extraction of the pigment and purification.

Sample	Absorbance[a]	Chlorophyll-a[b]	Final yield of chlorophyll-a [c]	Mg-yield[d]
C018	1.26 (20 ml)	0.31 ± 0.02 mg	86.0%	111.0%
C019	0.33 (37.2 ml)	0.15 ± 0.01 mg	86.1%	111.8%
C020	0.37 (60.5 ml)	0.28 ± 0.01 mg	91.5%	98.6%
C021	0.32 (62 ml)	0.25 ± 0.01 mg	85.3%	102.1%
C022	0.56 (47 ml)	0.33 ± 0.01 mg	93.3%	101.1%
C023	0.39 (57.8 ml)	0.28 ± 0.01 mg	72.5%	100.8%
D01	2.29 (71 ml)	1.98 ± 0.11 mg	61.8%	105.3%
D02	2.27 (68.5 ml)	1.89 ± 0.10 mg	92.5%	101.0%
D03	1.62 (112.5 ml)	2.22 ± 0.12 mg	89.1%	97.9%
D04	3.05 (106.5 ml)	3.93 ± 0.28 mg	50.7%	98.6%

[a]at 665.5nm (total volume of methanol extract);
[b]pigment extract in methanol ± 2σ;
[c]pure chlorophyll-a in methanol
[d]expressed as chlorophyll-bound Mg

Table 3: Magnesium-isotopic composition of standards, culture media and chlorophyll-a extracts.

Sample	$\delta^{26}Mg$[a] (‰) ± 2σ	$\delta^{25}Mg$[a] (‰) ± 2σ	$\Delta^{26}Mg$[b] (‰) ± 2σ	$\Delta^{25}Mg$[b] (‰) ± 2σ	N (R)[c]
DSM3	0 ± 0.038	0 ± 0.021			7 (1)
Cambridge 1	-2.527 ± 0.170	-1.304 ± 0.094			7 (1)
Cambridge 1[d]	-2.60 ± 0.14	-1.34 ± 0.07			35 (1)
Culture medium	-0.677 ± 0.194	-0.357 ± 0.101			9 (9)
$MgSO_4$ standard	-0.623 ± 0.153	-0.332 ± 0.055			3 (3)
before growth	-0.667 ± 0.239	-0.353 ± 0.121			3 (3)
after growth	-0.740 ± 0.171	-0.388 ± 0.118			3 (3)
S. elongatus Chlorophyll-a					
Early growth phase:	-1.389 ± 0.285	-0.724 ± 0.154	-0.712 ± 0.345	-0.366 ± 0.184	6 (6)
C018	-1.502 ± 0.036	-0.776 ± 0.022	-0.826 ± 0.198	-0.408 ± 0.104	1 (1)
C019	-1.377 ± 0.036	-0.718 ± 0.021	-0.700 ± 0.198	-0.361 ± 0.104	1 (1)
C020	-1.157 ± 0.038	-0.591 ± 0.022	-0.480 ± 0.198	-0.234 ± 0.104	1 (1)
C021	-1.510 ± 0.038	-0.806 ± 0.023	-0.834 ± 0.198	-0.448 ± 0.104	1 (1)
C022	-1.293 ± 0.043	-0.690 ± 0.024	-0.616 ± 0.199	-0.333 ± 0.104	1 (1)
C023	-1.494 ± 0.034	-0.772 ± 0.020	-0.817 ± 0.197	-0.414 ± 0.103	1 (1)
Late growth phase:	-1.208 ± 0.032	-0.618 ± 0.034	-0.531 ± 0.197	-0.261 ± 0.107	4 (4)
D01	-1.192 ± 0.034	-0.630 ± 0.019	-0.515 ± 0.197	-0.272 ± 0.103	1 (1)
D02	-1.205 ± 0.040	-0.597 ± 0.022	-0.528 ± 0.198	-0.239 ± 0.104	1 (1)
D03	-1.229 ± 0.040	-0.634 ± 0.020	-0.553 ± 0.198	-0.277 ± 0.103	1 (1)
D04	-1.205 ± 0.038	-0.612 ± 0.022	-0.529 ± 0.198	-0.255 ± 0.104	1 (1)
Spinach Chlorophyll-a[e]	-1.451 ± 0.098	-0.741 ± 0.062			-
Spinach Chlorophyll-b[e]	-2.352 ± 0.067	-1.204 ± 0.026			-

[a] The magnesium isotopic composition of the samples is expressed as a per-mil deviation from the DSM3 standard using the formula: $\delta^{x}Mg = \{(^{x}Mg/^{24}Mg)_{Sample}/(^{x}Mg/^{24}Mg)_{DSM3} -1\}$. [b] $\Delta^{x}Mg = \delta^{x}Mg_{sample} - \delta^{x}Mg_{culture\ medium}$. [c]N = number of replicates; R = number of separate samples. [d]Galy et al., (2003). [e]Young and Galy (2004).

Figure Captions:

Figure 1: Mg(II) is inserted into protoporphyrin IX via the magnesium-chelatase enzyme. The enzyme activity depends upon the hydrolysis of ATP to ADP, labilizing bound water from magnesium to enhance its rate of binding to the protoporphyrin IX precursor (Walker and Willows, 1997).

Figure 2: The growth of *S. elongatus* culture was monitored turbidimetrically using UV-vis spectra of the culture stocks. The absorbance at 750 nm varies with time according to the growth stage of the cyanobacterium. The stage of growth, at which samples were taken for isotopic analysis, are indicated as hollow circles on the black growth curve.

Figure 3: UV-Vis spectrum of various *S. elongatus* pigments in fractions separated on DEAE-Sepharose column. The dotted line corresponds to the spectrum of the methanol extract before pigment separation; the dashed and dotted line corresponds to the initial acetone elution of the cartenoids, such as zeaxanthin and β, β-carotene; the thin dashed line is an acetone rinse after collection of the cartenoid fraction; the thick solid line corresponds to the pure chlorophyll-a fraction; the thin solid line is a rinse after collection of the chlorophyll-a fraction.

Figure 4: Different ions are well separated by elution through the AG 50W-X12 cation-exchange column by 1.0 M HNO_3. The fraction corresponding to the Mg(II) ion was collected and prepared for isotopic analysis by evaporating and dissolving in 0.1 M HCl. The different lines correspond to individual columns of the same configuration that were used to separate Mg(II) from other ions in the broth and bacterium. The separations were clean and reproducible with a yield of ~100%.

Figure 5: Magnesium-isotope ratios expressed in per mil relative to the DSM3 standard using equation 1. The error bars correspond to ± 2σ. Figure 5a reports high precision, individual sample and standard measurements from this study. Some instrumental drift is observed as evidenced by repeated measurements of Cambridge 1. Figure 5b shows the average results of sample and standard measurements from this study compared with reports in the literature. There is a clear depletion of the heavy isoptopes of magnesium in chlorophyll-a relative to the culture medium from which the cyanobacteria were grown.

Figure 6: Structure of an average sized cell of *Synechococcus elongatus* showing the cell wall sheath (S) and cell membranes with cytoplasm membranes (Cm) separating the photosynthetic lamellae (Pl). The centre of the cell is filled with cytoplasm, ribosomes (R), DNA fibrils (D) and polyhedral bodies (Pb). Diagram adapted from SEM images of Gantt and Conti (1969).

Figure 1:

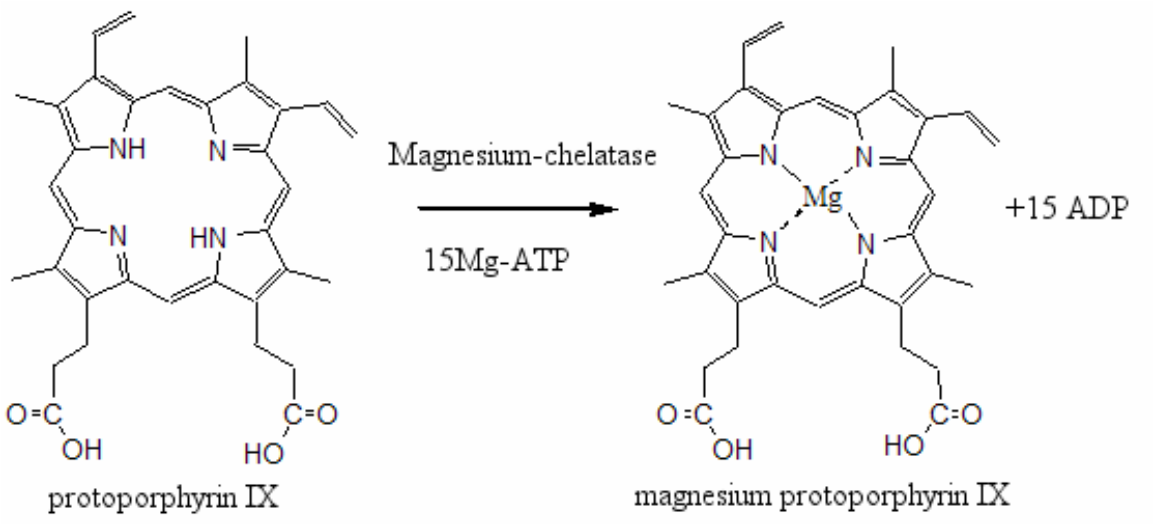

NH
N
N
HN
O=C
OH
C=O
HO
protoporphyrin IX
Magnesium-chelatase
15Mg-ATP
N
N
Mg
N
N
O=C
OH
C=O
HO
magnesium protoporphyrin IX
+15 ADP

Figure 2:

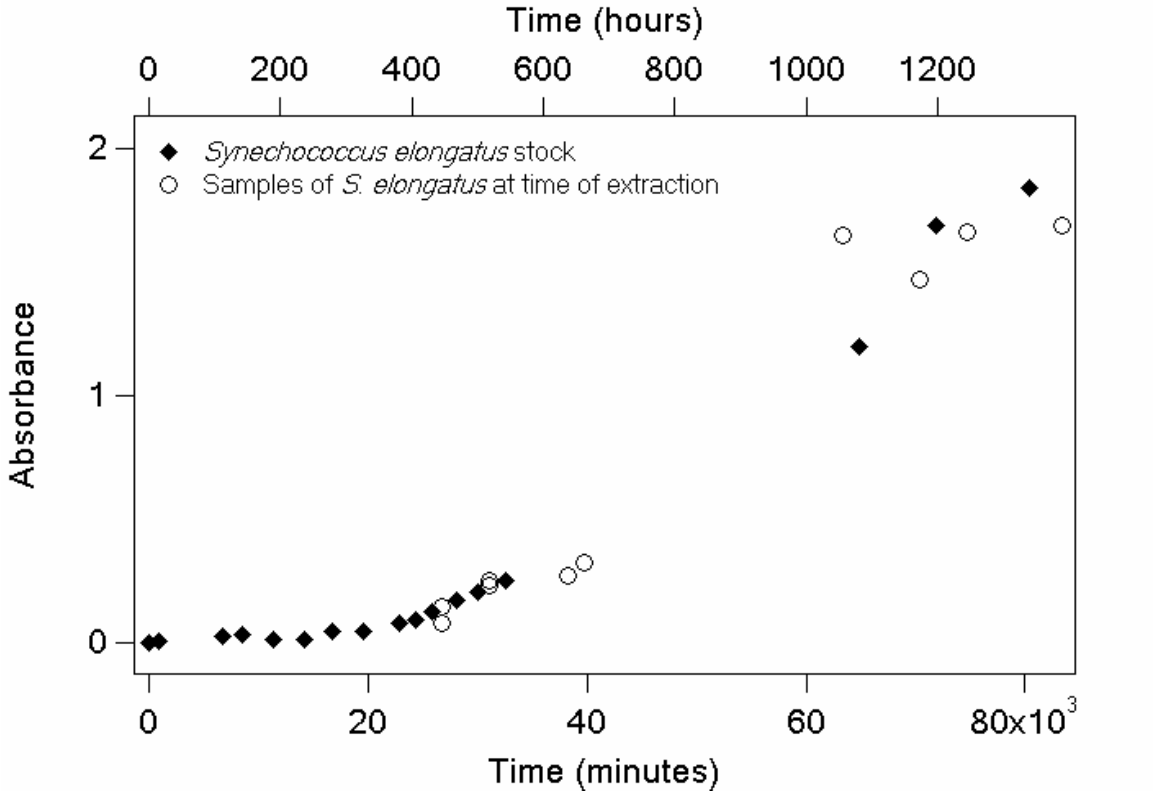

Time (hours)
0
200
400
600
800
1000
1200
Synechococcus elongatus stock
Samples of S. elongatus at time of extraction
Absorbance
2
1
0
0
20
40
60
80x10^3
Time (minutes)

Figure 3:

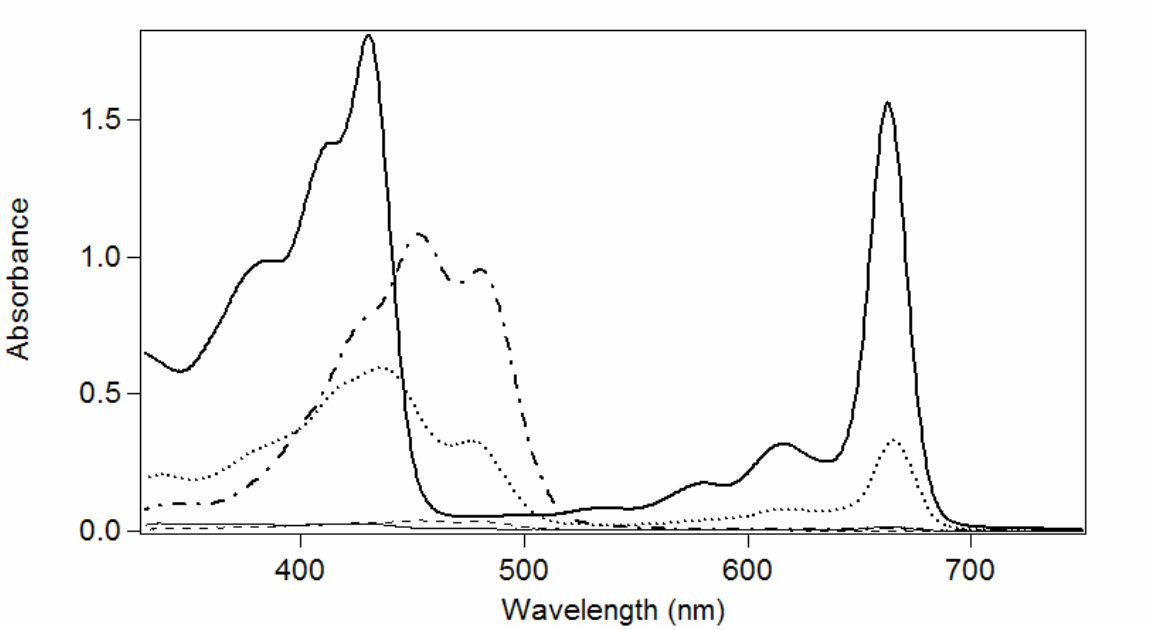


Figure 4:

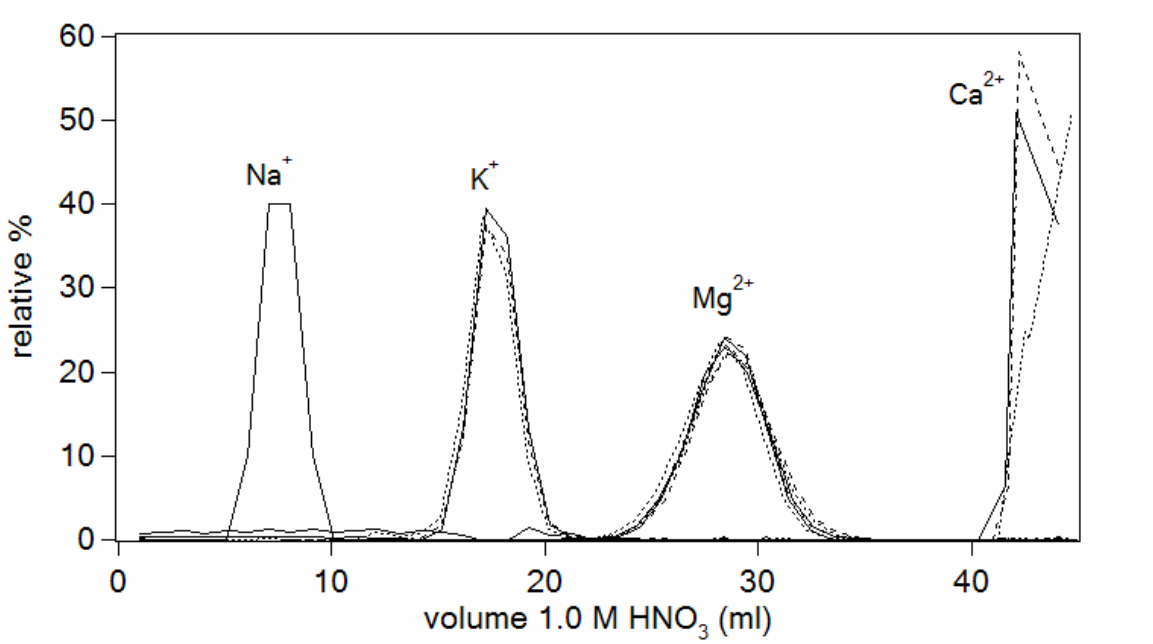

60
50
40
30
20
10
0
relative %
0
10
20
30
40
volume 1.0 M HNO3 (ml)
Na+
K+
Mg2+
Ca2+

Figure 5:

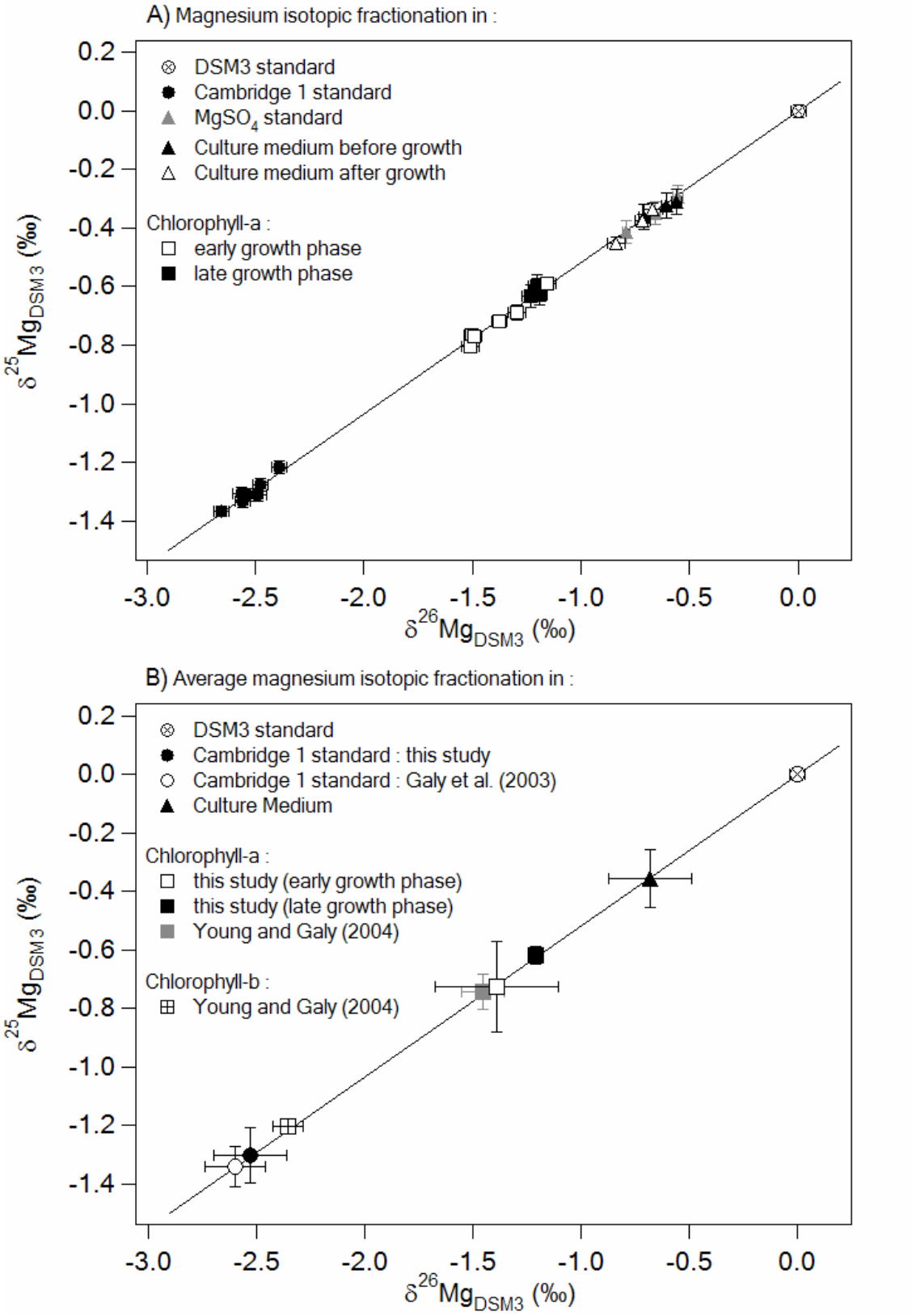
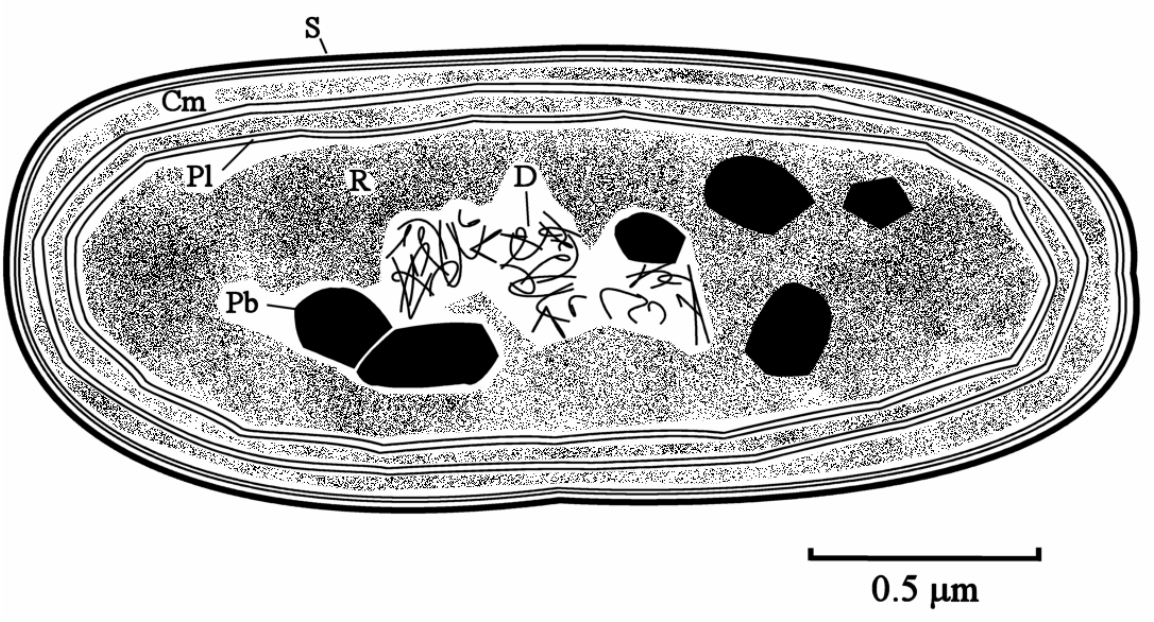

A) Magnesium isotopic fractionation in :
DSM3 standard
Cambridge 1 standard
MgSO4 standard
Culture medium before growth
Culture medium after growth
Chlorophyll-a :
early growth phase
late growth phase
δ25MgDSM3 (‰)
δ26MgDSM3 (‰)
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
-3.0
-2.5
-2.0
-1.5
-1.0
-0.5
0.0
B) Average magnesium isotopic fractionation in :
DSM3 standard
Cambridge 1 standard : this study
Cambridge 1 standard : Galy et al. (2003)
Culture Medium
Chlorophyll-a :
this study (early growth phase)
this study (late growth phase)
Young and Galy (2004)
Chlorophyll-b :
Young and Galy (2004)
δ25MgDSM3 (‰)
δ26MgDSM3 (‰)

Figure 6:



References

Allen M. M. (1968) Photosynthetic membrane system in Anacystis nidulans. *Journal of Bacteriology* **96**(3), 836-41.

Buchachenko A. L. (2001) Magnetic isotope effect: nuclear spin control of chemical reactions. *Journal of Physical Chemistry A* **105**(44), 9995-10011.

Buchachenko A. L., Kouznetsov D. A., Arkhangelsky S. E., Orlova M. A., and Markarian A. A. (2005a) Spin biochemistry: intramitochondrial nucleotide phosphorylation is a magnesium nuclear spin controlled process. *Mitochondrion* **5**(1), 67-69.

Buchachenko A. L., Kouznetsov D. A., Orlova M. A., and Markarian A. A. (2005b) Magnetic isotope effect of magnesium in phosphoglycerate kinase phosphorylation. *Proceedings of the National Academy of Sciences of the United States of America* **102**(31), 10793-10796.

Chang V. T. C., Makishima A., Belshaw N. S., and O'Nions R. K. (2003) Purification of Mg from low-Mg biogenic carbonates for isotope ratio determination using multiple collector ICP-MS. *Journal of Analytical Atomic Spectrometry* **18**(4), 296-301.

Falkowski P. G., Barber R. T., and Smetacek V. (1998) Biogeochemical controls and feedbacks on ocean primary productivity. *Science* **281**, 200-206.

Field C. B., Behrenfeld M. J., Randerson J. T., and Falkowski P. (1998) Biosphere: integrating terrestrial and oceanic components. *Science* **281**, 237-240.

Futai M. (1978) Experimental systems for the study of active transport in bacteria. In *Bacterial Transport*, Vol. 4, pp. 7-41. Marcel Dekker, Inc.

Galimov E. M. (1985) *The Biological Fractionation of Isotopes*. Academic Press, Inc.

Galy A., Belshaw N. S., Halicz L., and O'Nions R. K. (2001) High-precision measurement of magnesium isotopes by multiple-collector inductively coupled plasma mass spectrometry. *International Journal of Mass Spectrometry* **208**, 89-98.

Galy A., Yoffe O., Janney P. E., Williams R. W., Cloquet C., Alard O., Halicz L., Wadhwa M., Hutcheon I. D., Ramon E., and Carignan J. (2003) Magnesium isotope heterogeneity of the isotopic standard SRM980 and new reference materials for magnesium-isotope-ratio measurements. *Journal of Analytical Atomic Spectrometry* **18**(11), 1352-1356.

Gantt E. and Conti S. F. (1969) Ultrastructure of blue-green algae. *Journal of Bacteriology* **97**(3), 1486-1493.

Hansson M. and Kannangara C. G. (1997) ATPases and phosphate exchange activities in magnesium chelatase subunits of *Rhodobacter sphaeroides*. *Proceedings of the National Academy of Sciences United States of America* **94**, 13351-13356.

Jasper P. and Silver S. (1977) Magnesium transport in microorganisms. In *Microorganisms and Minerals*, Vol. 3 (ed. E. D. Weinberg), pp. 7-47. Marcel Dekker, Inc.

Kim D. W., Jeon B. K., Kang B. M., Lee N.-S., Ryu H.-I., and Lee Y.-I. (2003) Adsorption and isotope effects by ion exchange with 1-aza-12-crown-4 bonded Merrifield peptide resin. *Journal of Colloid and Interface Science* **263**(2), 528-532.

Kim D. W. and Kang B. M. (2001) Enrichment of magnesium isotopes by 2'-aminomethyl-15-crown-5 bonded Merrifield peptide resin. *Journal of Radioanalytical and Nuclear Chemistry* **250**(2), 291-294.

Kim D. W., Kang B. M., Jeon B. K., and Ryu H. (2002) Adsorption and isotope effects by ion exchange with aqueous-2-aminomethyl-18-crown-6 bonded Merrifield resin. *Journal of Colloid and Interface Science* **254**(1), 190-194.

Kuznetsov D. A., Arkhangelsky S. E., Berdieva A. G., Markaryan A. A., Khasigov P. Z., Gatagonova T. M., Ktsoeva S. A., and Orlova M. A. (2005) Stable isotopes of Mg^{2+} as activators of the suppressed ATP-generating function of mitochondria. *Biofizika* **50**(1), 80-85.

Omata T. and Murata N. (1980) A rapid and efficient method to prepare chlorophyll a and b from leaves. *Photochemistry and Photobiology* **31**(2), 183-5.

Porra R. J., Thompson W. A., and Kriedemann P. E. (1989) Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochimica et Biophysica Acta* **975**, 384-394.

Ra K. T., Masuzawa T., Shiraiwa Y., and Sawada K. (2003) Mg isotope ratios of chlorophylls of marine phytoplankton species. *Geochimica et Cosmochimica Acta* **67**(18 (Supplement 1)), A388.

Roman M. R., Adolf J. E., Bichy J., Boicourt W. C., Harding L. W. J., Houde E. D., Jung S., Kimmel D. G., Miller W. D., and Zhang X. (2005) Chesapeake bay plankton and fish abundance enhanced by Hurricane Isabel. *EOS* **86**(28), 261.

Silver S. and Walderhaug M. (1992) Gene regulation of plasmid- and chromosome-determined inorganic ion transport in bacteria. *Microbiological Reviews* **56**(1), 195-228.

Walker C. J. and Willows R. D. (1997) Mechanism and regulation of Mg-chelatase. *Biochemical Journal* **327**(2), 321-333.

Willows R. D. (2003) Biosynthesis of chlorophylls from protoporphyrin IX. *Natural Product Reports* **20**(3), 327-341.

Young E. D. and Galy A. (2004) The isotope geochemistry and cosmochemistry of magnesium. *Reviews in Mineralogy & Geochemistry* **55**, 197-230.