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Nonspecific uptake and homeostasis drive the oceanic cadmium cycle

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The global marine distributions of Cd and phosphate are closely correlated, which has led to Cd being considered as a marine micronutrient, despite its toxicity to life. The explanation for this nutrient-like behavior is unknown because there is only one identified biochemical function for Cd, an unusual Cd/Zn carbonic anhydrase. Recent developments in Cd isotope mass spectrometry have revealed that Cd uptake by phytoplankton causes isotopic fractionation in the open ocean and in culture. Here we investigate the physiochemical pathways that fractionate Cd isotopes by performing subcellular Cd isotope analysis on genetically modified microorganisms. We find that expression of the Cd/Zn carbonic anhydrase makes no difference to the Cd isotope composition of whole cells. Instead, a large proportion of the Cd is partitioned into cell membranes with a similar direction and magnitude of Cd isotopic fractionation to that seen in surface seawater. This observation is well explained if Cd is mistakenly imported with other divalent metals and subsequently managed by binding within the cell to avoid toxicity. This process may apply to other divalent metals, whereby nonspecific uptake and subsequent homeostasis may contribute to elemental and isotopic distributions in seawater, even for elements commonly considered as micronutrients.

biological fractionation | isotope geochemistry | metal homeostasis | subcellular analysis | trace metal

In addition to the macronutrients nitrate, phosphate, and silicate, marine phytoplankton require many essential metals to function correctly (1). The uptake and utilization of these micronutrients results in large vertical isotopic and concentration gradients for many transition metals in seawater (2). In the open ocean, Cd concentrations are as low as a few picomoles per kilogram (2, 3), with associated highly fractionated Cd isotopic compositions of up to several permil (Fig. 1) (4). In culture, phytoplankton consume small quantities of Cd (5–7) and exhibit light Cd isotope compositions (8). Taken together, the available data suggest that the marine geochemistry of Cd is dominated by nutrient-like processes (i.e., required uptake). However, only one biochemical function for Cd is known: CdCA1, a Cd/Zn carbonic anhydrase from the marine diatom *Thalassiosira weissflogii* (9, 10). Although ubiquitous in natural waters (11), CdCA1 is absent in numerous phytoplankton including coccolithophores, cyanobacteria, archaea, and several species of diatom (11) (Table S1), and it is thus debateable whether the expression of this single enzyme can account for the nutrient-like distribution of Cd in the global ocean. Here we examine the isotopic fractionation of Cd associated with CdCA1 that has been expressed *in vivo* by the model microorganism, *Escherichia coli*. Because *E. coli* has no inherent use for Cd, our experimental design permits comparison of cultures both overexpressing and not expressing CdCA1 in otherwise identical experiments. Using a systematic experimental approach, we are able to identify the general physiochemical pathways that fractionate Cd isotopes.

The role of CdCA1 expression in determining whole-cell isotopic compositions was investigated by introducing the CdCA1 coding sequence from *T. weissflogii* into the competent *E. coli* strain BL21(DE3). Seven replicate experiments were performed

that differed only in the final cell washing step (SI Materials and Methods and Table S2). In each experiment, the culture suspension was divided into two parallel cultures that were grown with and without the addition of isopropyl- β -D-1-thiogalactopyranoside (IPTG; a chemical inducer) to promote overexpression of CdCA1 (Fig. 2). By adding IPTG to only 7 of the 14 cultures, we were able to independently study the role of CdCA1 expression in determining whole-cell Cd isotopic compositions without complications arising from differing physiologies. The 14 cultures were grown for up to 4 h and harvested during the exponential growth stage.

Results

The whole-cell Cd isotope compositions in the 14 cultures from this study are lighter than the growth medium in experiments both with and without the expression of CdCA1. The weighted means of the two sets of cultures are $\alpha_{\text{cells-Cd(aq.)}} = 0.99960$ and $\alpha_{\text{cells-Cd(aq.)}} = 0.99957$ for induced and uninduced cultures, respectively (Fig. 2). The mean offset ($\alpha_{\text{cells-Cd(aq.)}} \sim 0.9996$) is in agreement with the existing, albeit limited (and lower precision), Cd isotope dataset for cultured freshwater phytoplankton ($\alpha_{\text{cells-Cd(aq.)}} = 0.9986 \pm 0.0006$; ref. 8) and the growing literature for other biologically active transition metals, whereby microorganisms exhibit a general preference for the light isotopes of a given element, e.g., Mo (12), Cu (13), Zn (14), Fe (15), and Ni (16). We rule out adsorption as the cause of the light isotopic fractionation observed for Cd in cultured cells, because a subset of cells plunged into the growth medium for a short time (~ 10 s) resulted in no fractionation of Cd isotopes (Fig. 2, control; SI Materials and Methods).

To probe the processes responsible for Cd isotopic fractionation, we dissected the *E. coli* cells into key subcellular components. The cells were first mechanically separated by sonication and centrifugation into cell membranes and the bulk cytosol. The cytosolic components were further separated by chromatography into the nonspecifically bound Cd (termed Cd ligand: CdL), Cd bound in CdCA1, and denatured CdCA1 (Fig. 3).

The subcellular separates exhibited a wide range of Cd isotope compositions (Table S3). The Cd bound in the cell membranes exhibited isotopic compositions negatively fractionated by $\delta^{114/110}\text{Cd}_{\text{membranes}} = -0.26 \pm 0.05$ compared with the Luria-Bertani (LB) growth medium; $\delta^{114/110}\text{Cd}_{\text{LB}} \equiv 0$] and accounted for $\sim 68\%$ of the total cellular burden. Cytosolic Cd ($\delta^{114/110}\text{Cd}_{\text{cytosol}} = -0.03 \pm 0.07$) is isotopically indistinguishable from the growth medium and contained the residual $\sim 32\%$ of the

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The authors declare no conflict of interest.

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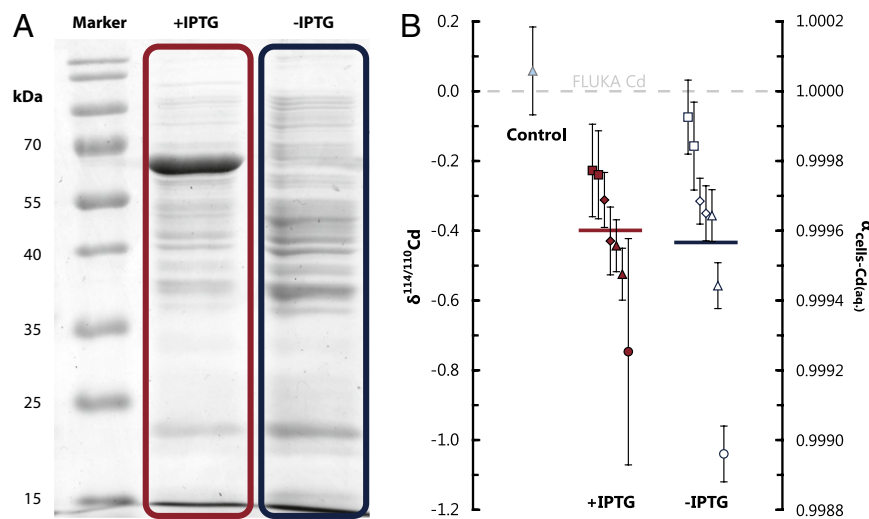


Fig. 2. CdCA1 expression does not affect whole cell Cd isotopic compositions. (A) Image of an SDS/PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis) separation of cellular proteins (stained with Coomassie brilliant blue R-250). CdCA1 (with histidine tag) expression is seen in the induced cultures (+IPTG) at 67.5 kDa (10) but not in the uninduced cultures (no IPTG). (B) Whole cell isotopic compositions for the control, induced, and uninduced cultures (with the means of the induced and uninduced cultures, weighted by 1/uncertainty, shown as the red and blue horizontal bars, respectively). The mean values of the +IPTG and -IPTG populations are indistinguishable ($P = 0.94$, using an unpaired two-sample t test). Isotopic data are reported relative to the Cd in the growth solution and as fractionation factors: $\alpha_{\text{cells-Cd(aq.)}}$. The control refers to cells that were harvested after ~ 10 -s exposure to Cd^{2+} . The spread in Cd isotopic compositions relates primarily to the cell washing tests ([SI Materials and Methods](#)). When cells washed with the same treatment are compared (same symbols), +IPTG and -IPTG cultures are within analytical uncertainty.

contend that metal homeostasis, rather than physiological function, drives the vertical isotopic (4) and concentration gradients (2, 3) of Cd seen in the global ocean. The fractionation observed with nonspecific uptake and homeostasis (i.e., membrane storage) in *E. coli* is consistent with the direction and magnitude of Cd isotopic fractionation for published seawater data (Fig. 1). Together, previous culturing studies and the genetic data are consistent with the nonspecific uptake and homeostasis mechanism proposed here, thus providing an alternative explanation as to the cause of the Cd-P association seen in the global ocean (2, 3).

In other words, all phytoplankton, regardless of their ability to use Cd, can contribute to the removal of Cd and fractionation of its isotopes in seawater (Fig. 1).

Nonspecific uptake and homeostasis may contribute to the distribution of other metals and their isotopic compositions in the oceans. Even metals with well-understood enzymatic roles (e.g., Fe) may exhibit a homeostasis control in regions of higher concentration (e.g., upwelling zones of the ocean). Despite the lack of similar subcellular analyses for other metal isotope systems, certain metal-specific storage proteins have been identified

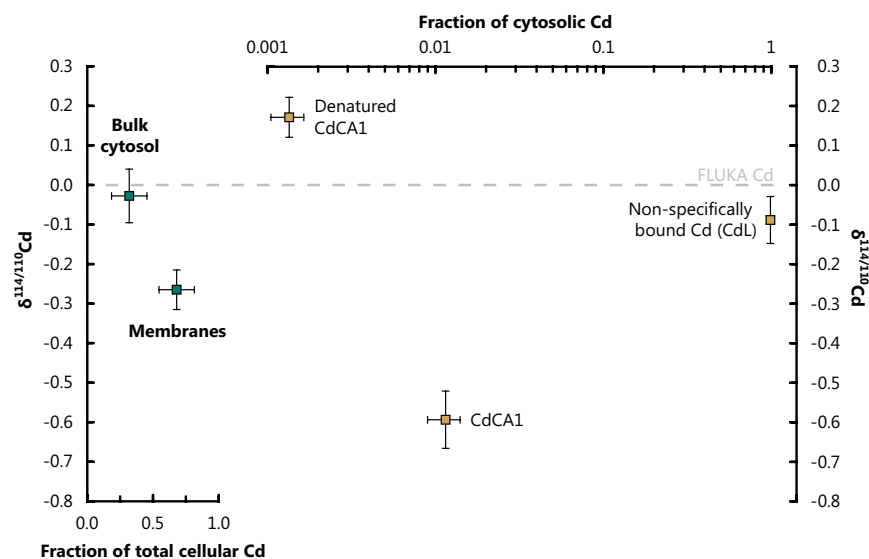
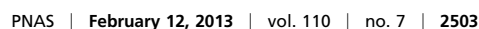


Fig. 3. Subcellular mass balance for *E. coli* cells expressing CdCA1. (*Left*) Isotopic composition of the cytosol (pre-separation) and cell membranes, demonstrating that the membranes dominate whole-cell isotopic compositions. (*Right*) Isotopic composition of the cytosolic components (post chromatographic separation); note the log scale. Nonspecifically bound Cd within the cytosol is isotopically identical to the bulk Cd isotopic composition of the cytosol and accounts for nearly all cytosolic Cd (~99%). CdCA1 is isotopically light by $\delta^{114/110}\text{Cd} = -0.59\text{‰}$, equivalent to $\alpha_{\text{CdCA1-cytosol}} = 0.9994 \pm 0.0001$, whereas denatured CdCA1 exhibits the only positively fractionated Cd within the cell.



method was robust in ensuring congruent dissolution and liberation of Cd from cell organic matter.

Decomposed samples were analyzed for Cd concentrations on a Thermo ELEMENT 2 ICPMS. A suitable mass of Cd double spike (^{111}Cd – ^{113}Cd) was then added, and the samples were purified using extraction chromatography (53, 54) in 200- μL shrink-fit polytetrafluoroethylene columns.

Isotopic Analysis of Cd. Isotopic methods have been reported in detail previously (55). Briefly, samples were introduced into a Nu Instruments DSN-100 desolvation system (at 120 $\mu\text{L}/\text{min}$) with an Elemental Scientific PFA MicroFlow Nebulizer. The desolvated sample was introduced into a Nu Instruments Nu Plasma multi-collector-ICPMS. All ion currents from 110 AMU (^{110}Cd , ^{110}Pd) to 117 AMU (^{117}Sn) were measured simultaneously in 40 \times 10-s integrations, using a MATLAB-based script to iteratively deconvolve sample isotopic compositions from the spike-sample mixture and isobaric interferences (^{112}Sn on ^{112}Cd , ^{113}In on ^{113}Cd , and ^{114}Sn on ^{114}Cd). Final isotopic compositions are reported using δ -notation: $\delta^{114/110}\text{Cd} = ([^{114}\text{Cd}/^{110}\text{Cd}]_{\text{sample}} / [^{114}\text{Cd}/^{110}\text{Cd}]_{\text{standard}} - 1) \times 1,000$, relative to the starting Cd in the growth solution, FLUKA Cd (Sigma-Aldrich CdCl₂, batch 20899). Because all Cd isotope compositions are reported relative to the Cd in the growth solution, the choice of reference

standard was unimportant. The total analytical blank was negligible in all cases at 8 ± 6 pg Cd (1 SD, $n = 7$), equivalent to $<0.1\%$ of the Cd present on any single isotopic measurement.

Where possible, uncertainties are quoted as $2 \times$ SD of sample replicates (when $n \geq 5$). When there was insufficient Cd to repeat the isotopic analyses at least five times, measurement uncertainties were derived from standard replicates, with similar Cd concentrations and spike/sample ratios that were run in the same analysis session, as this is generally a good approximation of the measurement uncertainty (53). (The uncertainty reported in the text is always the larger of the two.) The Cd isotopic composition of FLUKA Cd, relative to our in-house ICPMS standard OxCad (55), was determined as $\delta^{114/110}\text{Cd} = +0.75 \pm 0.10$ (2 SD, $n = 13$).

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- Morel FMM, Price NM (2003) The biogeochemical cycles of trace metals in the oceans. *Science* 300(5621):944–947.
- Bruland KW (1980) Oceanographic distributions of cadmium, zinc, nickel, and copper in the North Pacific. *Earth Planet Sci Lett* 47(2):176–198.
- Boyle EA, Sclater F, Edmond JM (1976) On the marine geochemistry of cadmium. *Nature* 263(5572):42–44.
- Ripperger S, Rehkämper M, Porcelli D, Halliday AN (2007) Cadmium isotope fractionation in seawater—A signature of biological activity. *Earth Planet Sci Lett* 261(3–4):670–684.
- Ho TY, et al. (2003) The elemental composition of some marine phytoplankton. *J Phycol* 39(6):1145–1159.
- Price NM, Morel FMM (1990) Cadmium and cobalt substitution for zinc in a marine diatom. *Nature* 344(6267):658–660.
- Xu Y, Tang D, Shaked Y, Morel FMM (2007) Zinc, cadmium, and cobalt interreplacement and relative use efficiencies in the coccolithophore *Emiliania huxleyi*. *Limnol Oceanogr* 52(5):2294–2305.
- Lacan F, Francois R, Ji Y, Sherrell RM (2006) Cadmium isotopic composition in the ocean. *Geochim Cosmochim Acta* 70(20):5104–5118.
- Lane TW, et al. (2005) Biochemistry: A cadmium enzyme from a marine diatom. *Nature* 435(7038):42.
- Xu Y, Feng L, Jeffrey PD, Shi Y, Morel FMM (2008) Structure and metal exchange in the cadmium carbonic anhydrase of marine diatoms. *Nature* 452(7183):56–61.
- Park H, Song B, Morel FMM (2007) Diversity of the cadmium-containing carbonic anhydrase in marine diatoms and natural waters. *Environ Microbiol* 9(2):403–413.
- Wasylenki LE, et al. (2007) Isotope fractionation during microbial metal uptake measured by MC-ICP-MS. *J Anal at Spectrom* 22(8):905–910.
- Navarrete JU, Borrok DM, Viveros M, Ellzey JT (2011) Copper isotope fractionation during surface adsorption and intracellular incorporation by bacteria. *Geochim Cosmochim Acta* 75(3):784–799.
- John SG, Geis RW, Saito MA, Boyle EA (2007) Zinc isotope fractionation during high-affinity and low-affinity zinc transport by the marine diatom *Thalassiosira oceanica*. *Limnol Oceanogr* 52(6):2710–2714.
- Beard BL, et al. (1999) Iron isotope biosignatures. *Science* 285(5435):1889–1892.
- Cameron V, Vance D, Archer C, House CH (2009) A biomarker based on the stable isotopes of nickel. *Proc Natl Acad Sci USA* 106(27):10944–10948.
- Waldron KJ, Robinson NJ (2009) How do bacterial cells ensure that metalloproteins get the correct metal? *Nat Rev Microbiol* 7(1):25–35.
- Waldron KJ, Rutherford JC, Ford D, Robinson NJ (2009) Metalloproteins and metal sensing. *Nature* 460(7257):823–830.
- Sunda WG (2012) Feedback interactions between trace metal nutrients and phytoplankton in the ocean. *Front Microbiol* 3:204.
- Twining BS, Baines SB (2013) The trace metal composition of marine phytoplankton. *Annu Rev Mar Sci* 5:191–215.
- Laddaga RA, Silver S (1985) Cadmium uptake in *Escherichia coli* K-12. *J Bacteriol* 162(3):1100–1105.
- Cullen JT, Sherrell RM (2005) Effects of dissolved carbon dioxide, zinc, and manganese on the cadmium to phosphorus ratio in natural phytoplankton assemblages. *Limnol Oceanogr* 50(4):1193–1204.
- Sunda WG, Huntsman SA (1996) Antagonisms between cadmium and zinc toxicity and manganese limitation in a coastal diatom. *Limnol Oceanogr* 41(3):373–387.
- Sunda WG, Huntsman SA (2000) Effect of Zn, Mn, and Fe on Cd accumulation in phytoplankton: Implications for oceanic Cd cycling. *Limnol Oceanogr* 45(7):1501–1516.
- Lane ES, Jang K, Cullen JT, Maldonado MT (2008) The interaction between inorganic iron and cadmium uptake in the marine diatom *Thalassiosira oceanica*. *Limnol Oceanogr* 53(5):1784–1789.
- Nies DH (1992) Resistance to cadmium, cobalt, zinc, and nickel in microbes. *Plasmid* 27(1):17–28.
- Finney LA, O'Halloran TV (2003) Transition metal speciation in the cell: Insights from the chemistry of metal ion receptors. *Science* 300(5621):931–936.
- Prévéral S, et al. (2009) A common highly conserved cadmium detoxification mechanism from bacteria to humans: heavy metal tolerance conferred by the ATP-binding cassette (ABC) transporter SpHMT1 requires glutathione but not metal-chelating phytochelatin peptides. *J Biol Chem* 284(8):4936–4943.
- Cobbett C, Goldsbrough P (2002) Phytochelatins and metallothioneins: Roles in heavy metal detoxification and homeostasis. *Annu Rev Plant Biol* 53:159–182.
- Silver S, Phung T (2005) A bacterial view of the periodic table: Genes and proteins for toxic inorganic ions. *J Ind Microbiol Biotechnol* 32(11–12):587–605.
- Grill E, Winnacker EL, Zenk MH (1987) Phytochelatins, a class of heavy-metal-binding peptides from plants, are functionally analogous to metallothioneins. *Proc Natl Acad Sci USA* 84(2):439–443.
- Mendoza-Cózatl DG, et al. (2010) Tonoplast-localized Abc2 transporter mediates phytochelatin accumulation in vacuoles and confers cadmium tolerance. *J Biol Chem* 285(52):40416–40426.
- Ortiz DF, Ruscitti T, McCue KF, Ow DW (1995) Transport of metal-binding peptides by HMT1, a fission yeast ABC-type vacuolar membrane protein. *J Biol Chem* 270(9):4721–4728.
- Lee JG, Ahner BA, Morel FMM (1996) Export of cadmium and phytochelatin by the marine diatom *Thalassiosira weissflogii*. *Environ Sci Technol* 30(6):1814–1821.
- Mitra RS, Gray RH, Chin B, Bernstein IA (1975) Molecular mechanisms of accommodation in *Escherichia coli* to toxic levels of Cd²⁺. *J Bacteriol* 121(3):1180–1188.
- Ahner BA, Morel FMM (1995) Phytochelatin production in marine algae. 2. Induction by various metals. *Limnol Oceanogr* 40(4):658–665.
- Dupont CL, Moffett JW, Bidigare RR, Ahner BA (2006) Distributions of dissolved and particulate biogenic thiols in the subarctic pacific ocean. *Deep Sea Res Part I Oceanogr Res Pap* 53(12):1961–1974.
- Ahner BA, Lee JG, Price NM, Morel FMM (1998) Phytochelatin concentrations in the equatorial pacific. *Deep Sea Res Part I Oceanogr Res Pap* 45(11):1779–1796.
- Ahner BA, Kong S, Morel FMM (1995) Phytochelatin production in marine algae. 1. An interspecies comparison. *Limnol Oceanogr* 40(4):649–657.
- Dupont CL, Ahner BA (2005) Effects of copper, cadmium, and zinc on the production and exudation of thiols by *Emiliania huxleyi*. *Limnol Oceanogr* 50(2):508–515.
- Marchetti A, et al. (2009) Ferritin is used for iron storage in bloom-forming marine pennate diatoms. *Nature* 457(7228):467–470.
- Dupont CL, Nelson RK, Bashir S, Moffett JW, Ahner BA (2004) Novel copper-binding and nitrogen-rich thiols produced and exuded by *Emiliania huxleyi*. *Limnol Oceanogr* 49(5):1754–1762.
- Totter S, et al. (2008) Protein-folding location can regulate manganese-binding versus copper- or zinc-binding. *Nature* 455(7216):1138–1142.
- Sunda WG, Huntsman SA (1995) Iron uptake and growth limitation in oceanic and coastal phytoplankton. *Mar Chem* 50(1–4):189–206.
- Kustka A, Sañudo-Wilhelmy S, Carpenter EJ, Capone DG, Raven JA (2003) A revised estimate of the iron use efficiency of nitrogen fixation, with special reference to the marine cyanobacterium *Trichodesmium* spp. (cyanophyta). *J Phycol* 39(1):12–25.
- Kustka AB, Allen AE, Morel FMM (2007) Sequence analysis and transcriptional regulation of iron acquisition genes in two marine diatoms. *J Phycol* 43(4):715–729.
- Saito MA, et al. (2011) Iron conservation by reduction of metalloenzyme inventories in the marine diazotroph *Crocospheera watsonii*. *Proc Natl Acad Sci USA* 108(6):2184–2189.
- Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227(5259):680–685.
- Sambrook J, Russell DW (2001) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, New York), p 2344.
- Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72:248–254.
- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. *J Mol Biol* 215(3):403–410.
- Larner F, et al. (2011) A new separation procedure for Cu prior to stable isotope analysis by MC-ICP-MS. *J Anal at Spectrom* 26(8):1627–1632.

