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Modulation of cadmium uptake in phytoplankton by seawater CO_2 concentration

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The vertical distribution of cadmium in the ocean is characteristic of an algal nutrient[1,2], although an underlying physiological basis remains undiscovered. The strong correlation between dissolved cadmium and phosphorus concentrations in sea water has nevertheless been exploited for reconstructing past nutrient distributions in the ocean[3–5]. In culture experiments, the addition of cadmium accelerates the growth of some marine phytoplankton[6–9] and increases the activity of carbonic anhydrase, normally a zinc-based metalloenzyme that is involved in inorganic carbon acquisition[7,9]. Here we show that the concentration of a Cd-carbonic-anhydrase—distinct from Zn-carbonic-anhydrases—in a marine diatom is regulated by the CO_2 partial pressure (p_{CO_2}) as well as by the zinc concentration. Field studies in intensely productive coastal waters off central California demonstrate that cadmium content in natural phytoplankton populations similarly increases as surface-water p_{CO_2} decreases. Incubation experiments confirm that cadmium uptake by natural phytoplankton is inversely related to seawater p_{CO_2} and zinc concentration. We thus propose that biological removal of cadmium from ocean surface waters is related to its utilization in carbonic anhydrase, and is regulated by dissolved CO_2 and zinc concentrations. The dissolved seawater Cd/P ratio would therefore vary with atmospheric p_{CO_2}, complicating the use of cadmium as a tracer of past nutrient concentrations in the upper ocean.

Previous work with laboratory cultures of several species of marine phytoplankton grown at low dissolved inorganic Zn concentrations has shown that Cd additions have a beneficial effect on growth rate, and that this effect is linked to an increase in carbonic anhydrase (CA) activity in the cells[6–9]. Further, at low inorganic Cd and Zn concentrations ($Cd' = 1$ pM and $Zn' = 3$ pM, where M′ indicates the sum of inorganic species) relevant to oceanic surface water[10,11], the uptake of Cd is increased with decreasing Zn (the usual metal cofactor in CA) and appears to be effected by a separate transport system[12]. CA, which catalyses the formation of CO_2 from HCO_3^-, is part of the inorganic carbon acquisition system whose activity is enhanced at low p_{CO_2} (ref. 7). We have now demonstrated that the diatom *Thalassiosira weissflogii* synthesizes a Cd-CA distinct from the other cellular Zn-CA (T.W.L. and F.M.M.M., unpublished results) as is illustrated in the native protein gels of Fig. 1 (lanes 6–9) showing distinct bands of ^{109}Cd and ^{65}Zn coeluting with CA activity. The Cd-CA is present in the cells even at an inorganic Zn concentration that allows maximum growth rate ($Zn' = 15$ pM; lanes 4–6), and its expression is regulated by p_{CO_2} as well as Zn′ (lanes 1–6). The relative amounts of ^{109}Cd present indicate that the concentration of Cd associated with the enzyme increases more than ten times when p_{CO_2} is decreased from 750 to 100 p.p.m. (lanes 1 and 3), and by about 50% when Zn′ is decreased from 15 to 3 pM (lanes 5 and 2).

As the phytoplankton Cd-CA is the only known biological function for Cd, its uptake by the biota in surface sea water may result from its use in such enzymes. We would then expect that Cd uptake by phytoplankton in the oceans should be modulated by ambient p_{CO_2} and Zn. We measured the Cd content of natural phytoplankton assemblages along environmental p_{CO_2} gradients during the April 1997 upwelling period within 50 km of the central California coast between Monterey Bay and Santa Barbara Channel (>0.45 μm particulate Cd/P ratios were determined directly by HR-ICP-MS (high resolution inductively coupled plasma mass spectrometry) $C/P = 106–120$, $n = 4$, for particulate samples from Monterey Bay, indicating that living biomass dominates particle composition). Surface-water p_{CO_2} varied from 250 p.p.m. in regional plankton blooms, found both in Monterey Bay and in the central Santa Barbara Channel, to >800 p.p.m. in recently upwelled water off Big Sur. The phytoplankton Cd/P ratio was inversely correlated to surface-water p_{CO_2}, with values for the >0.45-μm biomass varying from 0.06 to 0.7 mmol mol^{-1} over the sampled range of p_{CO_2} (Fig. 2). The lowest value, found in recently upwelled water, was six times lower than the dissolved Cd/P ratio in the upwelled water, while the highest—observed in regional high-chlorophyll blooms at $p_{CO_2} = 250–300$ p.p.m.—was twice the upwelled Cd/P ratio.

We also determined the Zn/P ratio of our samples; cellular Cd and Zn concentrations were inversely correlated, particularly in the large

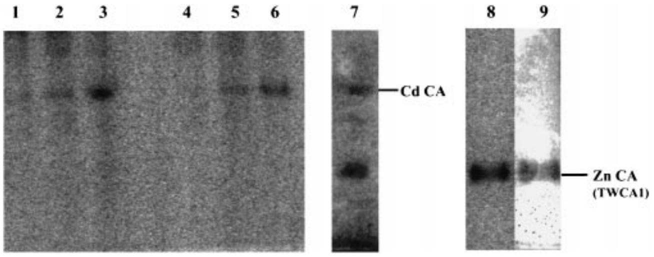

Figure 1 Modulation of Cd-carbonic-anhydrase (Cd-CA) by p_{CO_2} and Zn. Lanes 1–6 are phosphorimages of 10% native polyacrylamide gels of diatom proteins labelled with ^{109}Cd and grown under different concentrations of Zn and p_{CO_2}. Lanes 1–3, 3 pM Zn′, 30 pM Cd′; lanes 4–6, 15 pM Zn′, 30 pM Cd′; lanes 1 and 4, bubbled with 750 p.p.m. CO_2; lanes 2 and 5, 350 p.p.m. CO_2; lanes 3 and 6, 100 p.p.m. CO_2. Lane 7, CA activity assay of lane 6. Lane 8 is a phosphorimage of 10% native gel of diatom proteins labelled with ^{65}Zn grown with 15 pM Zn′ and 100 p.p.m. CO_2. Lane 9, CA activity assay of lane 8 (TWCA1 is the main Zn-containing isoform of CA in *T. weissflogii*). Under incubation conditions (20 °C, salinity = 30), p_{CO_2} values of 100, 350 and 750 p.p.m. correspond to aqueous concentrations of CO_2 of 3.3, 11.7, and 25.0 μM, respectively. *T. weissflogii* harvested in mid-exponential phase was resuspended in buffer (10 mM Tris-HCl, pH 7.2, 1% Triton X100) and lysed by sonication. Protein representing equal cell numbers was loaded in each lane of the gel. CA activity was detected by the bromothymol blue pH drift assay described elsewhere[27]. CA activity appears as dark on a lighter background. Relative amounts of radioactivity present in each band was determined by phosphorimaging.

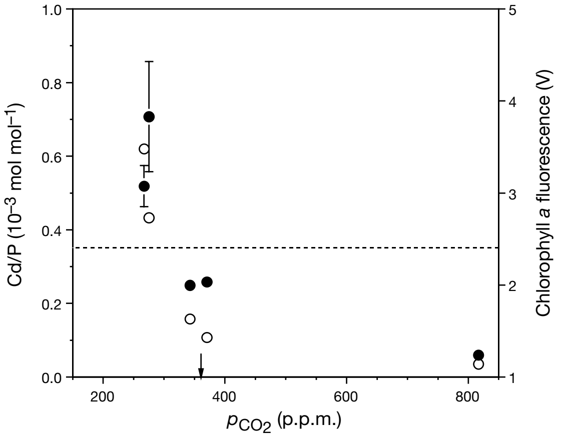

Figure 2 Phytoplankton Cd/P and chlorophyll *a* fluorescence versus p_{CO_2} in field samples. Fluorescence is reported as a relative analogue signal in volts (open circles). Samples were collected at locations within 50 km off central California in April 1997 (p_{CO_2} data courtesy of T. Takahashi, C. Sweeney and A. van Geen). The highest Cd/P ratio (>0.45 μm; closed circles) was observed in two bloom areas in Monterey Bay and Santa Barbara Channel. The calculated aqueous concentration of CO_2 at bloom sites reached a minimum of 12 μM. Error bars (some smaller than symbol) are estimated standard deviation of ratios, largely a function of filter blank reproducibility. Dotted line shows upwelled dissolved Cd/P ratio, and arrow indicates mean atmospheric p_{CO_2}. A novel sampling technique and analytical method was applied to measure particulate metals and P in field samples[28]. Surface-water samples collected with a trace-metal-clean pumping system were filtered through a series of three filters, ending with acid-leached 0.45-μm polysulphone filters (Supor, Gelman, Ann Arbor, Michigan). Samples were stored at −20 °C in the dark until analysed in the laboratory. Phytoplankton were digested in concentrated HNO_3 and HF acids (SeaStar, Sidney, British Columbia, Canada), and analysed for trace metals and P by HR-ICP-MS (Element, Finnigan-MAT, Bremen, Germany) using the method of standard additions. To calculate the composition of biogenic particles, Fe and Al were determined as tracers of inorganic particles to estimate non-biological fractions of metals and P in particulate samples. Based on average crustal ratios, terrigenous clays contributed <1% of total Cd, Zn and P in all samples discussed. Estimated P associated with authigenic Fe oxide phases was <5% of total measured P, based on P/Fe in Fe oxyhydroxides precipitated within hydrothermal plumes[29]. p_{CO_2} was determined by continuous (every 2.5 min) underway equilibrator measurements from ~2 m depth (T. Takahashi and C. Sweeney, personal communication).

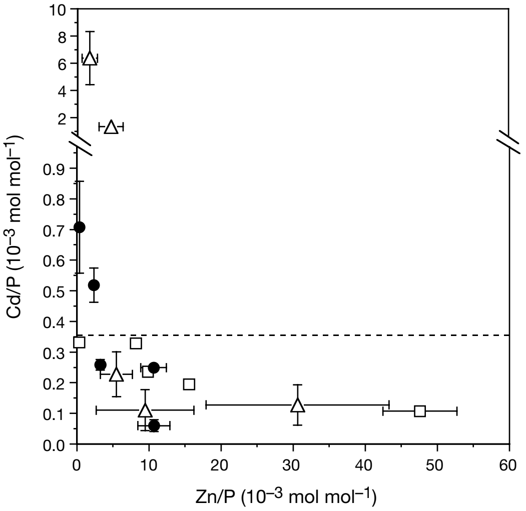

Figure 3 Cd/P versus Zn/P for phytoplankton off central California. Total >0.45 μm biomass (filled circles), 5–53 μm fraction (open squares), and >53 μm fraction (open triangles), for assemblages dominated by large chain-forming diatoms. Note the change in the scale to accommodate the very high Cd content of the largest size fraction in bloom regions.

size fractions (Fig. 3). The largest size fraction (>53 μm), which consisted primarily of large diatoms (mostly *Nitzschia* spp.) also had by far the highest Cd content in the bloom samples: Cd/P = 1–6 mmol mol^{-1} at 270 p.p.m. p_{CO_2}, ten times higher than in the same size fraction at 340–820 p.p.m. This result is significant as CO_2 uptake by large cells (>30 μm) may be diffusion-limited[13], implying increased requirement for CA at the dissolved CO_2 concentrations seen in our California bloom stations. The size-fractionated data provide evidence against adsorption of Cd on particles (living or dead) as a mechanism for removal, because larger particles with lower surface to volume ratios have higher, not lower, Cd/P ratios than smaller particles. As large, high-Cd diatoms account for a disproportionate fraction of export production[14–16], dissolved Cd may be removed preferentially to phosphate in productive, low-p_{CO_2} waters.

Because several chemical and biological parameters covary with p_{CO_2} in the surface waters off California, the observed correlations may not represent a causal dependency of particulate Cd on p_{CO_2} and Zn. To establish more directly the role of p_{CO_2} and Zn concentration in modulating Cd concentrations in marine phytoplankton, we performed short term (5–6 hours) ^{109}Cd and $H^{14}CO_3^-$ uptake experiments in shipboard incubations at varying Zn and p_{CO_2}, using the natural assemblage sampled from this region in May 1998. Calculated Cd/P (assuming the Redfield value of C/P = 106 mmol mol^{-1}) was substantially lower than in 1997 field samples. The reason for this difference is unknown, but may reflect a different flora or inhibition of Cd uptake by relatively high dissolved Mn concentrations in incubation seawater (40 nM, M. Wells, personal communication)[12]. This difference notwithstanding, the Cd content of the phytoplankton was found to depend on the concentrations of dissolved CO_2 and Zn. Cells acclimated to grow at 100 p.p.m. p_{CO_2} and ambient Zn (0.7 nM; M. Wells, personal communication) had intracellular Cd concentrations (measured as steady-state Cd/C uptake ratios) twice those of cells maintained at 350 and 800 p.p.m. CO_2 (Fig. 4). In all cases, we observed a significant decrease (50 ± 20%) in Cd uptake when 25 nM Zn was added to the samples. Nonetheless, at low p_{CO_2} (100 p.p.m.), the uptake of Cd was two or three times greater than at higher p_{CO_2}, even in the presence of a high Zn concentration.

The incubation experiments confirm that the variations in biological uptake of Cd in waters off California are indeed modulated by p_{CO_2} and Zn. Such modulation is consistent with the observed regulation of Cd-CA in culture, and supports the hypothesis that Cd uptake in surface sea water results from its utilization in Cd-CA in phytoplankton. On this basis, we predict that observed variations in upper-ocean Cd cycling reflect variations in ambient Zn and, particularly, CO_2 concentrations as observed in the waters off central California. For example, we suggest that high Cd uptake and vertical export can explain recent observations of sharply reduced dissolved Cd/P (0.05–0.15 mmol mol^{-1}; refs 17–19) in the low-p_{CO_2} (~100 p.p.m. undersaturated; ref. 20) subantarctic surface waters, compared to Cd/P (0.3 mmol mol^{-1}) in Antarctic waters south of the Polar Front[18,21,22]. Further, production of Cd-rich biomass is consistent with vertical profiles of dissolved Cd versus P north of the Polar Front, which show regeneration of organic matter containing high Cd/P (~0.5 mmol mol^{-1}; ref. 18).

A modulation of Cd uptake by p_{CO_2} also suggests that the biogeochemical cycle of Cd should respond to major changes in global climate. We postulate that the relationship between Cd and P in modern ocean surface waters is a result of dissolved CO_2 and Zn availability to large phytoplankton that dominate export production. Past changes in CO_2 and/or Zn availability may therefore have altered this relationship either globally or regionally. For example, at the low p_{CO_2} of the glacial atmosphere (~200 p.p.m.; ref. 23, 24), the p_{CO_2} of the subantarctic surface water (which may have been further

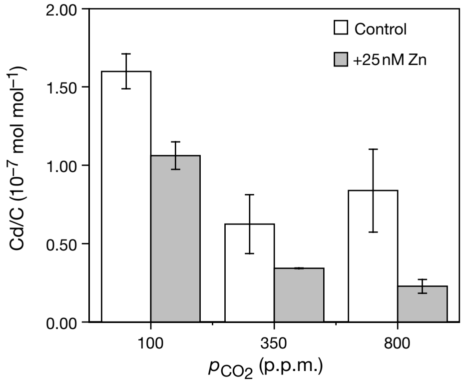

Figure 4 Cd/C uptake of phytoplankton grown at varying p_{CO_2} and [Zn(aq.)]. A natural phytoplankton assemblage from Monterey Bay was acclimated to three different p_{CO_2} levels at ambient (~0.7 nM) and amended Zn (+25 nM). Error bars represent propagated standard deviation based on duplicate measurements of Cd and C uptake rates. Surface-water samples (2-l bottles from 20-m depth) were collected in May 1998 and incubated on board ship using trace-metal-clean techniques at ambient sea surface temperature (13 ± 2 °C), bubbled with air–CO_2 mixtures at 100, 350 and 800 p.p.m. p_{CO_2} (Scott Specialty Gases, Plumsteadville, Pennsylvania). Following an acclimation period (20–27 h, ~1 doubling), samples were transferred to 250-ml acid-cleaned polycarbonate bottles (Nalgene), Zn was added to amendment bottles, and Cd and C uptake were measured in separate duplicate subsamples (250 ml) by short-term (5–6 h) uptake of added $\mathrm{H^{14}CO_3^-}$ and carrier-free ^{109}Cd (Zn and radioisotopes pre-equilibrated with 0.2-μm-filtered surface sea water during acclimation time). The total Cd addition to incubation bottles calculated from the specific activity of the primary stock was 10 pM. After gentle (<150 mm Hg) filtration onto 3-μm polycarbonate membrane filters, cells were rinsed with a 4 mM diethylenetriamine penta-acetic acid solution for 5 min, then washed with 0.2-μm-filtered sea water to remove extracellular surface adsorbed ^{109}Cd, and assayed for ^{109}Cd and ^{14}C activity by liquid scintillation counting. The Cd/C uptake ratio was determined using total inorganic carbon calculated for each p_{CO_2} level (at measured $T = 13$ °C, salinity = 33.135, and total alkalinity = 2,282 μequiv. kg^{-1}) and dissolved Cd = 0.7 nM throughout. Although C uptake (per chl *a*) varied with p_{CO_2} for both Zn levels, changes in C uptake were of secondary importance compared to variable Cd uptake in determining the differences observed in Cd/C ratios across treatments. p_{CO_2} levels of 100, 350 and 800 p.p.m. correspond to calculated aqueous CO_2 concentrations of 4, 14 and 32 μM, assuming solubility equilibrium.

undersaturated due to increased productivity during the Last Glacial Maximum, ref. 25) would have been <200 p.p.m., causing the preferential removal of Cd to be more intense than it is now. The resulting low dissolved Cd/P ratio in these waters would then have to be taken into account when interpreting Cd/Ca in fossil planktonic foraminifera as a proxy for surface-water phosphate concentrations[26]. Our results suggest that a region acting as a substantial atmospheric CO_2 sink probably exports Cd-rich organic matter, driving the surface water dissolved Cd/P ratio below the upwelled deep water ratio. If so, evidence for a lower glacial Cd/Ca ratio in planktonic foraminifera cannot be interpreted directly as more efficient surface-water nutrient utilization. □

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Influence of NO_x emissions from ships on tropospheric photochemistry and climate

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Emissions of nitrogen oxides (NO_x, the sum of NO and NO_2) from fossil-fuel burning dominate the NO_x burden of the lower troposphere in many regions[1]. These emissions increase tropospheric ozone and hydroxyl-radical concentrations over their natural 'background' levels, thereby increasing the oxidizing power of the atmosphere[2]. Fossil-fuel emissions of NO_x (refs 3, 4) account for about half of the global NO_x source to the atmosphere; other significant sources are from biomass burning[5], soil emissions[6], aircraft exhausts[7] and lightning[8], all primarily continental. However,