Mechanisms and Factors Regulating the Uptake and Toxicity of Heavy Metals in Phytoplankton

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LONG TERM GOALS

Our long-term research goals are to determine the influence of pollutant metals on the productivity and species composition of phytoplankton communities in coastal waters and to determine the role of algal metal uptake in controlling the particulate removal and biogeochemical cycling of metals.

OBJECTIVES

To achieve these goals, we need to determine the environmental factors and underlying physiological mechanisms that regulate the cellular accumulation and effects of toxic metals (Cu, Cd, and Zn) and interactive nutrient metals (Mn, Zn and Fe) in representative phytoplankton species. We then can combine this information with data on environmental variations in controlling factors (e.g., free ion concentrations of metals and specific growth rate) to construct conceptual and mathematical models for algal uptake, cellular accumulation, and biological effects of metals.

APPROACH

Our research approach involved conducting long-term growth experiments with algal cultures to quantify relationships among key dependent variables (growth rate, cellular metal concentrations, and steady-state metal uptake rates) and independent controlling variables (free ion concentrations of important metals and light); and (2) short-term kinetic studies to investigate physiological mechanisms of cellular metal uptake and binding characteristics of metal uptake systems. Many of our experiments examined metal/metal interactions, particularly those between toxic and nutrient metals. Effects of light were also examined because of its controlling influence on growth rate and biochemical demand for nutrient metals (Mn and Fe). The toxic metals investigated were Cu, Cd and Zn while the nutrient metals included Mn, Fe, and Zn. Zinc acts as either a nutrient or toxicant depending on its concentration. Free metal ion concentrations were controlled in these experiments by metal ion buffer systems employing the chelators EDTA or NTA. Cellular metal concentrations in long-term experiments and metal uptake rates in short-term experiments were measured with radiotracers ^{109}Cd, ^{65}Zn, ^{59}Fe, and ^{54}Mn.

Results from short-term experiments were modeled using competitive saturation kinetics to determine saturation uptake rates (V_{max}) and affinity constants for cellular metal uptake sites. These results were then combined with data from long term experiments (steady state cellular metal levels, metal uptake rates, and specific growth rate as functions of free metal ion concentrations) to yield conceptual and mathematical models of cellular metal uptake, cellular metal regulation, and metal effects on growth rate. The data from long term experiments were combined with field data for total and free ion

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concentrations of pertinent metals to predict algal removal of trace metals in coastal waters or metal effects on algal growth.

WORK COMPLETED

Much of the effort this year was spent in writing up results of specific studies for publication in peer reviewed journals (1 paper), writing synthesis papers which integrated findings from this project with existing ideas and data in the scientific literature (3 papers), and revision of papers that had been previously submitted (2 papers). The various papers are listed in the Publication section. An additional synthesis paper entitled "Bioavailability and bioaccumulation of iron in seawater," has recently been submitted for publication and is currently under review.

In addition, we conducted several series of long-term experiments examining the influence of light intensity and/or photoperiod on relationships among trace metal ion concentration, steady-state cellular metal uptake rate, cellular metal concentration, and specific growth rate for micronutrient metals Zn, Fe and Mn and the interactive toxic metal Cd, which is taken up by the cell's Mn transport system. The Zn and Fe experiments examined cellular response under both growth limiting and growth sufficient metal ion concentrations and involved the addition of single radiotracers (^{65}Zn or ^{59}Fe). Uptake and growth effects of Cd and Mn were examined in a dual-radiolabeled experiment conducted at high and low manganese ion concentrations, saturating and limiting light, and 7 Cd ion concentrations ranging from non-toxic to severely growth inhibiting. As part of our experiments with zinc and iron accumulation, we also examined diel variations in cellular metal concentrations and cellular metal:C ratios for cells grown under daily light:dark cycles.

RESULTS

Decreased light intensity or photoperiod increased cellular accumulation of Fe, Mn, and Cd in the coastal diatom *Thalassiosira pseudonana*. Previously, we had found that decreases in light intensity caused an increase in cellular Fe (Sunda and Huntsman 1997) and cellular Mn (Sunda and Huntsman in press) due to the increased metabolic need for Fe and Mn during cellular acclimation to reduced light. This increased need is related to the presence of both metals within key proteins or protein complexes within the photosynthetic apparatus. The observed increase in cellular iron was not related to an increase in cellular uptake rates as Fe uptake rates at high light appear to approach limits imposed by physical space on the outer cell membrane and inherent physicochemical limits on Fe ligand exchange kinetics. Thus, cells are unable to increase their iron uptake rates at low light to meet the increased metabolic demand, and as a result, iron uptake rates were the same at saturating light (500 µmol quanta m^{-2} s^{-1}) and growth limiting light (50 µmol quanta m^{-2} s^{-1}). However, at steady-state, the cellular metal concentration equals the uptake rate divided by the specific growth rate:

$$[\text{Cell metal}] = V_{Me} / \mu \qquad (1)$$

Thus, as growth rate decreased due to light limitation, the cellular Fe increased in inverse proportion the decrease in growth rate. Mn showed a similar pattern: its uptake rate was independent of light intensity, and consequently its cellular concentration also increased in inverse proportion to low-light induced decreases in growth rate. As the growth rate declined with light limitation, the cells used the higher accumulated levels of Fe and Mn to synthesize additional photosynthetic units needed for low light

acclimation. The increase in photosynthetic units was accompanied by a requisite increase in cellular chlorophyll concentrations.

In experiments conducted during the past year, we found that *T. pseudonana* cells respond to a shortened photoperiod in fundamentally the same way that they respond to decreased light intensity. When the photoperiod was shortened from 14 to 7 h d^{-1} under saturating light (500 µmol quanta m^{-2} s^{-1}), cellular growth rate decreased by 42%, the mean daily iron uptake rate remained constant, and consequently, mean daily cellular iron increased by 70% at a given concentration available inorganic iron species. In conjunction with the increase in cellular iron, chlorophyll increased by a similar amount (65%), apparently due to a photoacclimative increase in photosynthetic units.

At high zinc ion concentrations, Cd is taken up by the cell's Mn transport system (Sunda and Huntsman 1996), and consequently we would expect the pattern of uptake and accumulation of this metal under high and low light to resemble that of Mn. This prediction was verified in culture experiments with *T. pseudonana*. A decrease in light intensity from 500 to 50 µmol quanta m^{-2} s^{-1} resulted in a 2.9-fold decrease in growth rate, a similar 2.8-fold increase in both cellular Mn and chlorophyll, and a 2.2-fold increase in cellular Cd at low free Cd ion concentrations (0.1 to 1 nM). At higher ionic Cd, cellular Cd increased and the difference in cadmium content between low- and high-light acclimated cells narrowed to 36%. Modeling of data for cellular Cd, Mn and growth rate (see Sunda and Huntsman 1996) indicated that this narrowing of the differences in Cd content for low and high light cells was due to increased induction of Cd efflux in the low light cells which helps protect them from Cd poisoning. Cellular Mn uptake rates were independent of light intensity, but decreased by 450-fold as free Cd was increased from 10^{-10} to 10^{-7} M. This resulted in up to a 77-fold decrease in cellular Mn concentrations and a resultant induction of Mn deficiency and an associated 9-fold decrease in growth rate.

Variations in light intensity and photoperiod also affected cellular zinc concentrations and micronutrient limitation of growth rate, but the patterns were somewhat different from those with Fe and Mn. Unlike Fe and Mn, Zn is not directly involved in the light reactions of photosynthesis, but rather is needed for the supply of CO_2 to the Calvin Cycle in the dark reactions of photosynthesis. Consequently, as the carbon fixation rate decreases with decreasing light intensity, there is a concomitant decrease in the amount of cellular zinc needed to achieve maximum growth rate. For example at saturating light, an average daily Zn:C ratio of 11 µmol mol^{-1} was needed to achieve maximum growth rate (1.8 d^{-1}) while at a 10-fold lower light intensity, an average Zn:C of only 4 µmol mol^{-1} was needed to support the 3-fold lower maximum specific growth rate (0.6 d^{-1}). At low growth-limiting zinc ion concentrations of 1-3 pM, zinc uptake rates approached diffusion limiting values and were independent of decreases in growth rate caused by decreased light intensity or photoperiod. Under these conditions, the average Zn content of the cells at a given zinc ion concentration was inversely proportional to the specific growth rate. However, at growth-sufficient Zn ion concentrations of 30-100 pM, cellular Zn approached a constant regulated value that was independent of changes in external Zn^{2+} and growth rate. Under these regulated conditions, variations in growth rate were accompanied by concomitant variations in cellular Zn uptake rate.

As seen from Eq. 1, the concentration of metal accumulated within a cell represents a balance between the metal uptake rate and the specific growth rate. Equation 1 applies specifically to exponentially growing cells under steady state conditions. This relationship may not apply exactly, since cells usually experience a day:night cycle; and carbon fixation (on which growth depends) occurs only during

daylight, while metal uptake occurs during both day and night. Consequently, there can be an enhanced biodilution during the day, which can result in lower cell metal concentrations at the end of the light period. For example, in *Thalassiosira pseudonana* grown exponentially under a 7:17 h light:dark cycle (μ=1.0 d^{-1}), cellular zinc per liter of cell volume was 25% lower on average at the end of the light period than at the beginning. Cellular zinc:carbon ratios decreased even more (55%), due to two-fold increases in cell carbon to volume ratios, reflecting daytime storage of carbohydrates and subsequent utilization to support nighttime growth and metabolism. Similar, but less pronounced diel variations were observed for cellular Fe:C ratios. Although cellular metal concentrations and metabolic rates can vary during diel cycles, Eq. 1 does apply exactly if cellular metal concentrations, metal uptake rates and specific growth rates are all expressed as average daily values. This is important because of the simplicity and important predictive power of Eq. 1 (Sunda and Huntsman 1998).

IMPACT

Our results indicate that the uptake and toxicity of Cu, Cd, and Zn are not only controlled by the free ion concentrations of these metals, but also by the availability and cellular concentrations of nutrient metals (Mn, Zn and Fe) with which these metals competitively interact. Thus, factors that influence the cellular concentrations of Mn, Zn, and Fe can greatly influence whether or not a toxic metal will exert an inhibitory effect on algal growth rate or whether it is effectively removed from harbor waters by algal uptake and settling. Decreased light intensity or duration (daily photoperiod) increases cellular concentrations of Mn and Fe and, which should not only influence algal removal of these metals, but also influence uptake and effects of toxic metals with which these nutrient metals interact. For example, decreased light intensity increases the cellular accumulation of both Mn and Cd. Thus, Cd toxicity, trophic transfer, and biological removal should be increased in low light environments, such as found at the bottom of the euphotic zone, or in turbid, well-mixed harbor waters. Our work has shown fundamental links between metal uptake rates, cellular metal accumulation, and growth rate (see Eq. 1) in which decreases in growth rate can substantially increase metal accumulation if metal uptake rates remained fixed, which they often do since the rates for important nutrient metals (Fe and Zn) are often fixed by fundamental chemical and physical limits. Since algal cells experience diel variations in carbon fixation and growth due to diel light variations, metal to carbon ratios (e.g. Zn:C, Fe:C) also exhibit diel patterns, with highest ratios observed in the morning at the beginning of the light period. Such diel variability means that the time of day can be quite important to researchers interested in sampling metal concentrations in field plankton samples, and to agal grazers which may be exposed to higher metal contents in algal food consumed at in the morning than in similar food consumed during the evening.

RELATED PROJECTS

By combining our culture results with measurements of free ion concentrations of Cu, Zn, Cd, and Mn in estuarine and coastal waters provided by other contractors (Ken Bruland at UC, Santa Cruz; John Donat at Old Dominion and Jim Moffett at WHOI), we are beginning to formulate the theoretical framework for predicting or assessing the toxicity and biological scavenging of metals. Such modeling for Zn, for example, correctly predicts the extent of Zn removal from waters in Narragansett Bay. By contrast, there is a lack of algal removal of copper in that estuary and our models also correctly predict this observation. Observed lower than expected algal levels of the intracellular heavy metal binding agent phytochelatin, induced largely in response to cell Cd uptake, is readily explained by the measured high free ion concentrations of Mn and Zn, which markedly inhibit algal Cd uptake.

TRANSITIONS

Our results are being used to construct more realistic conceptual and numerical models of trace metal interactions with living cells in aquatic systems (e.g., see Watras 1998; Hudson 1998).

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