MEASUREMENT OF ELEMENT CONCENTRATIONS IN MARINE NANOPLANKTON CELLS USING AN X- RAY FLUORESCENCE MICROPROBE

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Abstract:
A novel technique is described for the quantification and localization of trace elements in marine nanoplankton that utilizes brilliant synchrotron x-rays to excite fluorescence. Thirteen individual cells, including ciliates, diatoms, and autotrophic flagellates collected off the coast of New York were analyzed for K, Ca, Fe, Cu, and Zn. There was considerable variability in trace metal concentrations both within and among taxa from a single site. This technique for studying the distribution of elements within single cells may provide a unique understanding of metal-biota interactions.

key words: synchrotron; metals; plankton

Introduction

The development of "clean" techniques has led to a sharp increase in studies on the geochemistry of trace metals and their roles as limiting nutrients for oceanic primary production. These studies require the use of bulk size-fractionation techniques to isolate organisms or particles from their surrounding liquid medium for analysis. Typically, whole water is collected and filtered either serially or in parallel through 0.2, 2.0 and 20 μm membrane filters to divide the particles into the corresponding biological size classes of pico-, nano-, and microplankton (1). The filters are then rinsed and analyzed for metal content. This approach can only provide information about the metal contents of the trapped particles in aggregate. In situations where there is a mixed assemblage of microbial organisms (as is usually the case in natural waters), this "bulk chemistry" technique cannot distinguish metal concentrations in different organisms of the same size or between living and abiotic particles. Additionally, heterogeneity among cells of the same species is not assessed, as all of the particles in a certain size class are grouped together for analysis.

Materials and methods

We have attempted to overcome the limitation of bulk- chemistry metal analysis through the use of a high-brilliance synchrotron x-ray fluorescence microprobe at the Advanced Photon Source (APS) at Argonne National Laboratory. This instrument uses highly-focused x-rays to induce fluorescence in metal atoms within individual plankton cells (2). Natural plankton samples are mounted on EM grids, and individual cells of interest are identified either through light or epifluorescence microscopy. The grids are then mounted in the x-ray microprobe and the same cells identified and characterized by light microscopy are targeted and scanned with the x-ray beam. The optics of the x-ray beam can be adjusted to increase photon flux (increasing sensitivity) or to reduce the area of the beam (increasing resolution). At each pixel in the two dimensional scan, the elements present in the sample are excited by the incident x-ray beam and emit fluorescent photons at characteristic energies, producing a fluorescence spectra for each pixel detected by germanium lithium detectors. The fluorescence intensity is quantified and converted to metal concentration through the use of standards. The technique is similar to energy-dispersive x-ray fluorescence used to quantify elements in macrophyte algae (3). Without the brilliant x-rays provided by the synchrotron, however, this technique lacks the sensitivity to measure metals in single-celled plankton samples. New third-generation synchrotrons such as the APS provide the necessary x-ray intensity. Additionally, advances in x-ray optics have reduced the size of the incident x-ray beam, increasing the resolution of the instrument to 0.2 μm (or 0.04 μm^2). The microprobe has the sensitivity to quantify and map Si, Ca, K, Fe, Cu, and Zn (and potentially Cr, Mn, Ni, As, and Se) in individual planktonic particles. The high resolution allows us to quantify element concentrations in different particles and to map the distribution of metals within single particles. We have used this novel technique to quantify elemental concentrations in several different classes of nanoplankton collected off the coast of Southampton, NY. In order to present the results as cellular concentrations, cellular metal contents were normalized to biomass by assuming a squashed ellipsoidal cell shape in combination with published estimates of C:volume ratio for each type of cell (4-6) and assuming C:dry weight conversions of 3 for ciliates and flagellates and 4 for diatoms. We examined two oligotrichous Strombidium-like ciliates 15 μm in diameter, 6 large Thalassiosira-like centric diatoms (25 μm diameter), and 5 small autotrophic flagellates (6 μm diameter).

Results and discussion

The measured concentrations of K, Ca, Fe, Cu, and Zn in ciliates, diatoms and flagellates are shown in Table 1. We included K and Ca in our results as possible indicators of cellular biomass. These are the first reported results, to our knowledge, of metal concentrations in individual phytoplankton and protozoa cells. All of the samples were collected from the same site and the ciliate and diatom cells appear to be of the same genus, respectively. Although these results are based on a small sample size, they suggest that there is considerable variation in the metal concentrations of similar cells from the same location as well as between the different cell types. The diatoms show the highest metal concentrations of all the cells, and the flagellates have significantly lower concentrations than the two groups of larger cells. The concentrations of Fe, Cu, and Zn in the ciliates and flagellates are within the range of values reported for plankton from bulk chemical measurements taken during a diatom bloom off Monterey, CA (7), but the diatoms in the present study have Fe and Cu concentrations that are 135% and 231% higher, respectively. Our results might be expected to be higher since the other study included all plankton-sized particles, including detritus that may have had lower metal concentrations.

Table 1. Concentrations of five metals in marine ciliates (n = 2 cells examined), diatoms (n = 6 cells), and flagellqates (n = 5 cells). Mean values (µg g-1 dry wt) and standard deviations (SD) are given.

Element	Ciliates (n=3)	SD	Diatoms (n=6)	SD	Flagellates (n=5)	SD
K	16,781	20,857	35,759	21,533	1,546	934
Ca	8,264	2,772	19,772	16,527	2,397	1,413
Fe	424	117	809	575	87	40
Cu	337	140	693	183	88	111
Zn	106	0	295	134	37	22

This technique also shows promise as a tool for studying the internal distribution of metals within individual plankton cells. Fig. 1 shows the distributions of 6 elements in one of the diatom cells. The elemental concentrations vary spatially: Si is primarily found in the exterior test of the cell; Cl, Ca, and Zn are more common in the organic biomass of the cell, as shown by their density in the two frustules of the cell; Fe appears to be highly localized in the upper frustule.

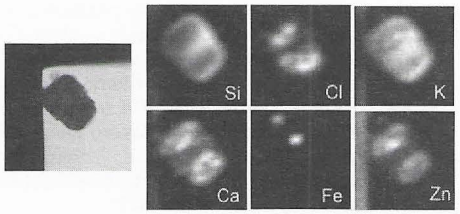

Fig. 1: The first image (right) is a light micrograph of a diatom. The other six images show the distribution of Si, Cl, K, Ca, Fe, and Zn within the same cell, based on x-ray fluorescence.

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