Partitioning of iron and plutonium in exopolymeric substances and intracellular biopolymers: a comparison study between the coccolithophore Emiliania huxleyi and the diatom Skeletonema costatum

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Project

» Biopolymers as carrier phases for selected natural radionuclides (of Th, Pa, Pb, Po, Be) in diatoms and coccolithophores (Biopolymers for radionuclides)

Contributors	Affiliation	Role
Santschi, Peter	Texas A&M, Galveston (TAMUG)	Principal Investigator
Quigg, Antonietta	Texas A&M, Galveston (TAMUG)	Co-Principal Investigator
Schwehr, Kathleen	Texas A&M, Galveston (TAMUG)	Co-Principal Investigator
Xu, Chen	Texas A&M, Galveston (TAMUG)	Co-Principal Investigator
Biddle, Mathew	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract

Iron (Fe), a micronutrient for algal growth, and plutonium (Pu), an anthropogenic radionuclide, share some common features. This includes similar oceanic distributions when different input modes are taken into account, as well as their chemical behavior, such as a high affinity to natural organic matter (NOM). The NOM produced by various phytoplankton communities can potentially influence Fe cycling in the ocean, and likely also influence the transport behavior of Pu. We conducted laboratory incubation experiments using the coccolithophore Emiliania huxleyi and the diatom Skeletonema costatum, in the presence of 59Fe and 238Pu as radiotracers, in order to differentiate Fe and Pu uptake by extracellular exopolymeric substances (EPS) and intracellular biopolymers. The Fe and Pu distributions in select organic compound classes including proteins, total carbohydrates (TCHO) and uronic acids (URA) produced by these two types of phytoplankton were compared. Our results indicated that most of the Fe and Pu (>95%) were found concurrently concentrated in E. huxleyi-derived non-attached EPS, while much less (<2%) was present in the intracellular fraction of E. huxleyi. By contrast, in the diatom S. costatum, both Fe and Pu distribution was EPS > intracellular biopolymers > outer cell covering (i.e., frustule). In fact, over 50% of Fe was concentrated in S. costatum-derived attached EPS and intracellular biopolymers. The diatom derived Fe-EPS complexes were more hydrophobic, with stronger tendency to aggregate in seawater. Fe binding to biopolymers in both E. huxleyi and S. costatum cultures was related to URA concentrations, but the overall distribution of URA between these two phytoplankton species was different. Our findings suggest that the presence of URA in S. costatum cellular surface (i.e., attached EPS) and its intracellular fraction could be an indicator for the Fe transport from the surrounding seawater to the diatom cells. However, for the coccolithophore E. huxleyi, Fe appeared not to be efficiently taken up during its growth. Instead, the more hydrophilic non-attached EPS (i.e., low protein/TCHO ratio) produced by E. huxleyi could have stabilized Fe in the colloidal form as Fe-EPS complexes. Similar partitioning behavior of Fe and Pu suggests that Pu isotopes can potentially serve as a tracer for the Fe biogeochemistry in the ocean.

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Dataset Description

Iron (Fe), a micronutrient for algal growth, and plutonium (Pu), an anthropogenic radionuclide, share some common features. This includes similar oceanic distributions when different input modes are taken into account, as well as their chemical behavior, such as a high affinity to natural organic matter (NOM). The NOM produced by various phytoplankton communities can potentially influence Fe cycling in the ocean, and likely also influence the transport behavior of Pu. We conducted laboratory incubation experiments using the coccolithophore Emiliania huxleyi and the diatom Skeletonema costatum, in the presence of 59Fe and 238Pu as radiotracers, in order to differentiate Fe and Pu uptake by extracellular exopolymeric substances (EPS) and intracellular biopolymers. The Fe and Pu distributions in select organic compound classes including proteins, total carbohydrates (TCHO) and uronic acids (URA) produced by these two types of phytoplankton were compared. Our results indicated that most of the Fe and Pu (>95%) were found concurrently concentrated in E. huxleyi-derived non-attached EPS, while much less (<2%) was present in the intracellular fraction of E. huxleyi. By contrast, in the diatom S. costatum, both Fe and Pu distribution was EPS > intracellular biopolymers > outer cell covering (i.e., frustule). In fact, over 50% of Fe was concentrated in S. costatum-derived attached EPS and intracellular biopolymers. The diatom derived Fe-EPS complexes were more hydrophobic, with stronger tendency to aggregate in seawater. Fe binding to biopolymers in both E. huxleyi and S. costatum cultures was related to URA concentrations, but the overall distribution of URA between these two phytoplankton species was different. Our findings suggest that the presence of URA in S. costatum cellular surface (i.e., attached EPS) and its intracellular fraction could be an indicator for the Fe transport from the surrounding seawater to the diatom cells. However, for the coccolithophore E. huxleyi, Fe appeared not to be efficiently taken up during its growth. Instead, the more hydrophilic non-attached EPS (i.e., low protein/TCHO ratio) produced by E. huxleyi could have stabilized Fe in the colloidal form as Fe-EPS complexes. Similar partitioning behavior of Fe and Pu suggests that Pu isotopes can potentially serve as a tracer for the Fe biogeochemistry in the ocean.

Acquisition Description

The seawater (< 1 kDa) was enriched with f/2 nutrients, trace metals and vitamins, and autoclaved in pre-combusted and seawater-preconditioned clear glassware. Known activity of 59Fe (gamma emitting radionuclide) and 238Pu (alpha emitting radionuclide) were added into the seawater in pre-combusted and seawater-preconditioned clear glassware.

After checking the pH of each radiolabeled medium to be 8.0, laboratory axenic Skeletonema costatum (UTEX LB 2308) and Emiliania huxleyi (CCMP 371) was added to 100 mL of media and incubated at a temperature of 19±1ºC with a light:dark cycle of 14 h:10 h under an irradiation condition of 100 µmol-quanta/m2/s.

The sequential chemical extraction scheme for obtaining individual fractions from S. costatum and E. huxleyi followed the procedures described in Chuang et al. (2015) and Lin et al. (2017), with a few exceptions. For the extracellular biopolymers excreted by the phytoplankton, non-attached exopolymeric substances (NAEPS) in the surrounding seawater and attached EPS (AEPS) associated with cellular surface, were harvested. Laboratory cultures were centrifuged at 3000 x g for 30 min, followed by filtration of the supernatant which was further concentrated and desalted with nanopure water (18.2 Ω) in 3 kDa Microsep centrifugal filter tubes (Milipore) to obtain the NAEPS fraction, while the resultant pellet from the centrifugation was resuspended by 50 mL 3% NaCl solution and stirred gently overnight at 4ºC to extract EPS from the cellular surface. The solution was also centrifuged, and the supernatant containing the AEPS was then filtered to remove residual cells before further desalting via the 3 kDa ultrafiltration centrifugation tubes. The final volume of concentrated solution of each biopolymer fraction (>3 kDa) was 2 mL.

For the S. costatum cultures, 10 mL of 100 mM EDTA (pH 8.0) solution was added to the diatom cells from the previous AEPS extraction step. The diatom cells were resuspended at 4ºC overnight to extract the intracellular material after diatom cell lysis and the supernatant was collected after centrifugation to obtain the EDTA-extractable intracellular biopolymers. Then, the resultant pellet was further resuspended in 10 mL of 1% SDS/10 mM Tris (pH 6.8) solution and heated at 95ºC for 1 hr. The centrifuged supernatant was also collected and defined as SDS-extractable biopolymer in S. costatum cells.

To access the diatom frustule-associated biopolymers, 5 mL of 52% HF was then added to the frustules and incubated on ice for 1 hr. After the separation of HF-insoluble pellet, the HF-soluble fraction was evaporated under N2 stream and neutralized, followed by the 3 kDa centrifugal filtration to collect the digested frustule silica fraction (<3 kDa) and HF-soluble frustule-associated biopolymer (>3 kDa). Lastly, the residue biopolymer in the HF-insoluble pellet was collected with the resuspension in a 2 mL of 100 mM ammonium acetate solution and sonication. Similar to NAEPS and AEPS, all the S. costatum cellular biopolymers were concentrated and desalted with nanopure water in 3 kDa Microsep centrifugal filter tubes (Milipore).

The coccosphere of the E. huxleyi cells was first dissolved before the extraction of intracellular biopolymers. In brief, the pellet from the previous AEPS extraction step was digested in 0.44 M acetic acid (HAc) (weak acidity and non-oxidizing nature to avoid the breakage of cells) plus 0.1 M NaCl solution at 4ºC for 8 hr. After the digestion, the mixed solution was centrifuged and filtered, followed by ultrafiltration of the supernatant with 3 kDa Microsep centrifugal filter tubes. The retentate (>3 kDa) was defined as coccosphere-associated biopolymers, and the permeate fraction (<3 kDa) was also collected to obtain the

fraction of digested biogenic calcite.
The E. huxleyi cells after the removal of shells were further heated in 20 mL of 1% SDS/10 mM Tris mixed solution (pH 6.8) at 95 ºC for 1 hr. The supernatant was also collected through centrifugation and filtration, followed by desalting with 3 kDa Microsep centrifugal filter tubes. Subsequently, the remaining pellet was further digested by 0.04 M $NH_2OH•HCl$/4.35 M HAc mixture at 96 ºC for 6 hr to obtain the intracellular metabolitic biopolymer. The sum of these two fractions represents the intracellular biopolymers in E. huxleyi cells.
All the solutions from the different extraction steps, including the >3 kDa biopolymer fractions and the permeate (< 3 kDa, i.e., frustule and coccosphere), were counted to determine the activity of 59Fe and 238Pu. 59Fe activity was directly obtained from a Canberra ultrahigh purity germanium well gamma detector at the decay energies of 1099 kev. All the solutions for the gamma counting had the same volume and geometry to avoid geometry corrections, and all the data were decay corrected.
238Pu activities were determined by alpha-spectroscopy (Xu et al., 2016). Briefly, a known activity of 242Pu was spiked to trace the yield of 238Pu during the extraction steps. The samples were oven-dried, then heated at 600 ºC overnight in a ceramic crucible. The resulting ash fraction was then digested in Teflon tubes overnight in concentrated HNO_3 and HCl (1:1) at 85ºC. The remaining solid residual fraction was collected by centrifugation and discarded, and the supernatant was further evaporated to incipient dryness. To convert all Pu ions to Pu(IV), a $FeSO_4•7H_2O$ (0.2 g/mL) solution, followed by 0.25 g of $NaNO_2$, were added to each sample to achieve a final volume of 3 mL for each sample. Samples were then passed through an UTEVA column (Cat. # UT-C50-A, Eichrom, USA) to separate Pu from other alpha-emitting radionuclides (e.g., 238U, 241Am). After washing the column with an 8 M HNO_3 solution, the Pu was eluted using freshly-prepared 0.02 M $NH_2OH•HCl$/0.02 M ascorbic acid in 2 M HNO_3. The Pu-containing eluent was evaporated and re-constituted in 0.4 M $(NH_4)_2SO_4$ (pH~2.6) for electroplating onto a stainless steel planchet at 0.6 Amps current for 2 hr. Sample-bearing planchets were then analyzed via alpha spectroscopy for at least one week to obtain counting errors (1 sigma) lower than 5%.

Subsamples were taken from the concentrated biopolymers for the analysis of protein, total carbohydrate (TCHO) and uronic acid (URA), respectively. In brief, the protein abundance was measured through a modified Lowry protein assay, using bovine serum albumin (BSA) as the standard. For the concentrations of TCHO, samples were hydrolyzed by 0.09 M HCl (final concentration) at 150ºC for 1 h. After neutralization with NaOH solution, the hydrolysate was measured by the 2,4,6-tripyridyl-triazine (TPTZ) method (Hung et al., 2001), with glucose as the standard. URA concentrations were determined by the metahydroxyphenyl method using glucuronic acid as the standard (Hung and Santschi, 2001).

Processing Description

Microsoft Excel Ver. 15.15; KaleidaGraph Ver.4.1.3

BCO-DMO Processing Notes:
- added conventional header with dataset name, PI name, version date
- modified parameter names to conform with BCO-DMO naming conventions
- combined the two spreadsheets on type and Biopolymer_fraction

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Related Publications

Chuang, C.-Y., Santschi, P. H., Xu, C., Jiang, Y., Ho, Y.-F., Quigg, A., … Schumann, D. (2015). Molecular level characterization of diatom-associated biopolymers that bind234Th,233Pa,210Pb, and7Be in seawater: A case study withPhaeodactylum tricornutum. Journal of Geophysical Research: Biogeosciences, 120(9), 1858–1869. doi:10.1002/2015JG002970

Hung, C.-C., & Santschi, P. H. (2001). Spectrophotometric determination of total uronic acids in seawater using cation-exchange separation and pre-concentration by lyophilization. Analytica Chimica Acta, 427(1), 111–117. doi:10.1016/S0003-2670(00)01196-X

Hung, C.-C., Tang, D., Warnken, K. W., & Santschi, P. H. (2001). Distributions of carbohydrates, including uronic acids, in estuarine waters of Galveston Bay. Marine Chemistry, 73(3-4), 305–318. doi:10.1016/S0304-4203(00)00114-6

Lin, P., Xu, C., Zhang, S., Sun, L., Schwehr, K. A., Bretherton, L., … Santschi, P. H. (2017). Importance of coccolithophore-associated organic biopolymers for fractionating particle-reactive radionuclides (234 Th, 233 Pa, 210 Pb, 210 Po, and 7 Be) in the ocean. Journal of Geophysical Research: Biogeosciences, 122(8), 2033–2045. doi:10.1002/2017JG003779

Xu, C., Zhang, S., Chuang, C., Miller, E. J., Schwehr, K. A., & Santschi, P. H. (2011). Chemical composition and relative hydrophobicity of microbial exopolymeric substances (EPS) isolated by anion exchange chromatography and their actinide-binding affinities. Marine Chemistry, 126(1-4), 27–36. doi:10.1016/j.marchem.2011.03.004

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Parameters

Parameter	Description	Units
type	type	unitless
Biopolymer_fraction	Biopolymer fraction type	unitless
Cell_type	cell type	unitless
Fe59_act_pcnt	Activity percentage	unitless (%)
Pu238_act_pcnt	Activity percentage	unitless (%)
Protein	amount of protein	microMole Carbon (uM-C)
TCHO	amount of TCHO-total carbohydrate	microMole Carbon (uM-C)
URA	amount of URA-uronic acid	microMole Carbon (uM-C)
Protein_C_TCHO_C	amount of protein to total carbohydrates	microMole Carbon (uM-C)
pcnt_URA_TCHO	percent uronic acid to total carbohydrates	microMole Carbon (uM-C)

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Instruments

Dataset-specific Instrument Name	Canberra Quad Alpha Spectrometer Model 7404
Generic Instrument Name	Spectrometer
Dataset-specific Description	Sample-bearing planchets were then analyzed via alpha spectroscopy for at least one week to obtain counting errors (1 sigma) lower than 5%.
Generic Instrument Description	A spectrometer is an optical instrument used to measure properties of light over a specific portion of the electromagnetic spectrum.

Dataset-specific Instrument Name	UV-Visible spectrometer, BioTek Instruments Inc Model EPOCH
Generic Instrument Name	Spectrometer
Dataset-specific Description	UV-Visible spectrometer, BioTek Instruments Inc Model EPOCH
Generic Instrument Description	A spectrometer is an optical instrument used to measure properties of light over a specific portion of the electromagnetic spectrum.

Dataset-specific Instrument Name	Beckman Coulter Allegra X-12 centrifuge
Generic Instrument Name	Centrifuge
Dataset-specific Description	Beckman Coulter Allegra X-12 centrifuge
Generic Instrument Description	A machine with a rapidly rotating container that applies centrifugal force to its contents, typically to separate fluids of different densities (e.g., cream from milk) or liquids from solids.

Dataset-specific Instrument Name	Canberra ultrahigh purity germanium well gamma detector Model GCW3024
Generic Instrument Name	Gamma Ray Spectrometer
Dataset-specific Description	Canberra ultrahigh purity germanium well gamma detector Model GCW3024
Generic Instrument Description	Instruments measuring the relative levels of electromagnetic radiation of different wavelengths in the gamma-ray waveband.

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Project Information

Biopolymers as carrier phases for selected natural radionuclides (of Th, Pa, Pb, Po, Be) in diatoms and coccolithophores (Biopolymers for radionuclides)

NSF Award Abstract: Particle-associated natural radioisotopes are transported to the ocean floor mostly via silica and carbonate ballasted particles, allowing their use as tracers for particle transport. Th(IV), Pa (IV,V), Po(IV), Pb(II) and Be(II) radionuclides are important proxies in oceanographic investigations, used for tracing particle and colloid cycling, estimating export fluxes of particulate organic carbon, tracing air-sea exchange, paleoproductivity, and/or ocean circulation in paleoceanographic studies. Even though tracer approaches are considered routine, there are cases where data interpretation or validity has become controversial, largely due to uncertainties about inorganic proxies and organic carrier molecules. Recent studies showed that cleaned diatom frustules and pure silica particles, sorb natural radionuclides to a much lower extent (by 1-2 orders of magnitude) than whole diatom cells (with or without shells). Phytoplankton that build siliceous or calcareous shells, such as the diatoms and coccolithophores, are assembled via bio-mineralization processes using biopolymers as nanoscale templates. These templates could serve as possible carriers for radionuclides and stable metals. In this project, a research team at the Texas A & M University at Galveston hypothesize that radionuclide sorption is controlled by selective

biopolymers that are associated with biogenic opal (diatoms), CaCO3 (coccolithophores) and the attached exopolymeric substances (EPS), rather than to pure mineral phase. To pursue this idea, the major objectives of their research will include separation, identification and molecular-level characterization of the individual biopolymers (e.g., polysaccharides, uronic acids, proteins, hydroquinones, hydroxamate siderophores, etc.) that are responsible for binding different radionuclides (Th, Pa, Pb, Po and Be) attached to cells or in the matrix of biogenic opal or CaCO3 as well as attached EPS mixture, in laboratory grown diatom and coccolithophore cultures. Laboratory-scale radiolabeling experiments will be conducted, and different separation techniques and characterization techniques will be applied. Intellectual Merit : It is expected that this study will help elucidate the molecular basis of the templated growth of diatoms and coccoliths, EPS and their role in scavenging natural radionuclides in the ocean, and help resolve debates on the oceanographic tracer applications of different natural radioisotopes (230,234Th, 231Pa, 210Po, 210Pb and 7,10Be). The proposed interdisciplinary research project will require instrumental approaches for molecular-level characterization of these radionuclides associated carrier molecules. Broader Impacts: The results of this study will be relevant for understanding biologically mediated ocean scavenging of radionuclides by diatoms and coccoliths which is important for carbon cycling in the ocean, and will contribute to improved interpretation of data obtained by field studies especially through the GEOTRACES program. This new program will enhance training programs at TAMUG for postdocs, graduate and undergraduate students. Lastly, results will be integrated in college courses and out-reach activities at Texas A&M University, including NSF-REU, Sea Camp, Elder Hostel and exhibits at the local science fair and interaction with its after-school program engaging Grade 9-12 students from groups traditionally underrepresented.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1356453

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