Activity percent and composition percent of radionuclides, protein, carbohydrates, and iron at various pH levels using isoelectric focusing of radionuclide-labeled EPS

Website: https://www.bco-dmo.org/dataset/764608
Data Type: experimental
Version: 1
Version Date: 2019-04-10

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
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Abstract
Laboratory studies were conducted to examine the sorption of selected radionuclides (234Th, 233Pa, 210Po, 210Pb, and 7Be) onto inorganic (pure silica and acid-cleaned diatom frustules) and organic (diatom cells with or without silica frustules) particles in natural seawater and the role of templating biomolecules and exopolymeric substances (EPS) extracted from the same species of diatom, Phaeodactylum tricornutum, in the sorption process. The range of partition coefficients (Kd, reported as logKd) of radionuclides between water and the different particle types was 4.78–6.69 for 234Th, 5.23–6.71 for 233Pa, 4.44–5.86 for 210Pb, 4.47–4.92 for 210Po, and 4.93–7.23 for 7Be, similar to values reported for lab and field determinations. The sorption of all radionuclides was significantly enhanced in the presence of organic matter associated with particles, resulting in Kd one to two orders of magnitude higher than for inorganic particles only, with highest values for 7Be (logKd of 7.2). Results further indicate that EPS and frustule-embedded biomolecules in diatom cells are responsible for the sorption enhancement rather than the silica shell itself. By separating radiolabeled EPS via isoelectric focusing, we found that isoelectric points are radionuclide specific, suggesting that each radionuclide binds to specific biopolymeric functional groups, with the most efficient binding sites likely occurring in acid polysaccharides, iron hydroxides, and proteins. Further progress in evaluating the effects of diatom frustule–related biopolymers on binding, scavenging, and fractionation of radionuclides would require the application of molecular-level characterization techniques.

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Dataset Description

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Methods & Sampling

Diatom cultures, sample preparation, and EPS extraction
P. tricornutum (UTEX 646) was selected for culturing in autoclaved f/2 and f/2-Si media (salinity of 26) at a temperature of 19 + 1 oC with a light cycling of 14 h : 10 h under a saturating irradiance of 100 umol quanta m-2 s-1. In order to deplete the diatom of Si supply, cultures were transferred into f/2-Si medium over at least six generations by harvesting cells (2694 g, 30 min) and resuspending them in fresh f/2-Si medium. Sterile polycarbonate bottles were also used to prevent Si supply from glassware. The growth status of P. tricornutum was monitored by changes in optical density at 750 nm. Cells, frustules, and EPS were collected when P. tricornutum reached the stationary phase.

Laboratory cultures of P. tricornutum were centrifuged (2694 x g, 30 min) and filtered (0.2 um) to collect the whole cells. The frustules were repeatedly treated by using a hydrogen peroxide (30%, room temperature) treatment until bubbles were no longer generated, followed by concentrated nitric acid (HNO3) digestion (85oC, 1 h) to remove organic matter adopted from Robinson et al. (2004).

The resulting organic carbon (C), nitrogen (N), and sulfur (S) contents of the cleaned frustules were measured using a Perkin Elmer CHNS 2400 analyzer to ensure the removal of organic materials using cysteine as a standard according to Guo and Santschi (1997).

EPS extraction was followed the procedures described in Xu et al. (2011b), which minimize cell rupture and molecular alterations and maximize extraction efficiency. EPS here is referring to those biopolymers that are attached on the diatom frustules. Hereafter, EPS Si+ and EPS Si2 denote the EPS extracted from diatoms cultured under Si-replete (f/2 medium) and Si-depleted (f/2-Si medium) conditions, respectively. Briefly, laboratory cultures were centrifuged (2694 x g, 30 min) and filtered (0.2 um) when diatoms reached stationary phase. The diatom cells were soaked with 0.5 mol L-1 sodium chloride (NaCl) solution for 10 min and followed by centrifugation at 2000 x g for 15 min to remove the medium and weakly bound organic material on the cells. The pellet from previous step was resuspended in a new 100 mL 0.5 mol L-1 NaCl solution and stirred gently overnight at 4oC. The resuspended particle solution was ultracentrifuged at 12,000 x g (30 min, 4uC), and the supernatant was then filtered through a 0.2 um polycarbonate membrane. The filtrate was desalted and collected with a 1 kDa cutoff cross-flow ultrafiltration and diafiltration membrane and then freeze-dried for later use.

Characterization of exopolymeric substances

After partitioning EPS collected from lab cultures into aliquots for freeze-drying, subsamples were analyzed for individual components. Concentration of total carbohydrate (TCHO) concentration was determined by the TPTZ (2,4,6-tripyridyl-s-triazine) method using glucose as the standard, and uronic acids were measured by the meta-hydroxyphenyl method using glucuronic acid as the standard (Hung and Santschi 2001). Protein content was determined using a modified Lowry protein assay, using bovine serum albumin (BSA) as the standard (Pierce, Thermo Scientific). C, N, and S contents were determined as described above. Iron was measured using an atomic absorption spectrometer (Varian) after overnight digestion with 12 mol L^{-1} HNO3 at 85^{o}C (Von Loon 1985). To evaluate the protein size distribution pattern in EPS Si+ and EPS Si2, sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to Sambrook et al. (1989) using standard molecular weight markers (Dual Xtra Standards, Bio-Rad).

Fourier transform infrared spectroscopy (FTIR) was used to characterize samples using a Varian 3100 model interfaced with a single reflection horizontal attenuated total reflectance (ATR) accessory (PIKE Technologies). A diamond plate was used as the internal reflection element. A freeze-dried EPS sample was mounted at the surface of the diamond. Absorbance spectra from 800 to 2000 cm21 were collected and integrated using Varian Resolution Pro 4.0 software. ATR-FTIR spectroscopy provides a noninvasive way to quickly gain information about the contents of major secondary structures of biopolymers (Xu et al. 2011b; Jiang et al. 2012). Major infrared (IR) peaks were assigned according to Xu et al. (2011b) and Jiang et al. (2012). Characteristic bands found in the IR spectra of proteins and polypeptides include the amide I (1652–1648 cm^{-1}) and amide II (1550–1548 cm^{-1}) band. The absorption associated with the amide I band leads to stretching vibrations of the C=O bond of the amide, and absorption associated with the amide II band leads primarily to bending vibrations of the N-H and C-N bond. The symmetric stretching peak due to deprotonated carboxyl groups is observed at 1400 cm^{-1} along with the CH_2 bending mode at 1455 cm^{-1}. In the 800–1200 cm^{-1} regions, responses from C-O, C-O-C, P-O-P, C-O-P, and ring vibrations of the main polysaccharide functional groups are present in polysaccharide mixtures. The peaks at 1241 and 1113 cm^{-1} correspond to P-O stretching in phosphate groups.

Isoelectric focusing of radionuclide-labeled EPS

EPS Si+ and EPS Si2 were incubated separately with 234Th, 233Pa, 210Pb, 210Po, and 7Be, respectively, for subsequent IEF electrophoresis separation to determine the pHIEF of selected radionuclide binding ligands (Alvarado Quiroz et al. 2006). Briefly, radiolabeled biopolymers and 140 mL of rehydration solution were loaded onto an immobilized pH gradient strip (General Electric Healthcare Immobiline Drystrip, pH 3–10, 11 cm) and were reswelled overnight. Afterward, the strip was loaded into the device for isoelectric focusing for 17.5 h. The strip was then cut into 11 1 cm pieces and followed by 1% SDS extraction overnight. Five radionuclide activities of each fraction were subsequently analyzed. Due to the limited amount of each strip fraction, selected chemical compositions (TCHO, proteins, and Fe) of individual fraction were characterized as described above.

Data Processing Description

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 submitted tables together on the pH column, indicating percent activity and percent composition in the parameter names.

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Data Files

File	
Isoelectric_Focusing_electrophoresis.csv	(Comma Separated Values (.csv), 1.43 KB) MD5:a0eff7fd0ce7cec12d76f5557181c607
Primary data file for dataset ID 764608	

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Related Publications

Alvarado Quiroz, N. G., Hung, C.-C., & Santschi, P. H. (2006). Binding of thorium(IV) to carboxylate, phosphate and sulfate functional groups from marine exopolymeric substances (EPS). Marine Chemistry, 100(3-4), 337–353. doi:10.1016/j.marchem.2005.10.023
Methods

Baskaran, M., & Santschi, P. H. (1993). The role of particles and colloids in the transport of radionuclides in

coastal environments of Texas. Marine Chemistry, 43(1-4), 95–114. doi:10.1016/0304-4203(93)90218-d https://doi.org/10.1016/0304-4203(93)90218-D
Methods

Chuang, C.-Y., Santschi, P. H., Ho, Y.-F., Conte, M. H., Guo, L., Schumann, D., … Li, Y.-H. (2013). Role of biopolymers as major carrier phases of Th, Pa, Pb, Po, and Be radionuclides in settling particles from the Atlantic Ocean. Marine Chemistry, 157, 131–143. doi:10.1016/j.marchem.2013.10.002
Methods

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Results

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Methods

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Methods

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Methods

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 https://doi.org/10.1016/S0003-2670(00)01196-X
Methods

Jiang, Y., Yoshida, T., & Quigg, A. (2012). Photosynthetic performance, lipid production and biomass composition in response to nitrogen limitation in marine microalgae. Plant Physiology and Biochemistry, 54, 70–77. doi:10.1016/j.plaphy.2012.02.012
Methods

Quigley, M. S., Santschi, P. H., Hung, C.-C., Guo, L., & Honeyman, B. D. (2002). Importance of acid polysaccharides for234Th complexation to marine organic matter. Limnology and Oceanography, 47(2), 367–377. doi:10.4319/lo.2002.47.2.0367
Methods

Roberts, K. A., Xu, C., Hung, C.-C., Conte, M. H., & Santschi, P. H. (2009). Scavenging and fractionation of thorium vs. protactinium in the ocean, as determined from particle–water partitioning experiments with sediment trap material from the Gulf of Mexico and Sargasso Sea. Earth and Planetary Science Letters, 286(1-2), 131–138. doi:10.1016/j.epsl.2009.06.029
Methods

Robinson, R. S., Brunelle, B. G., & Sigman, D. M. (2004). Revisiting nutrient utilization in the glacial Antarctic: Evidence from a new method for diatom-bound N isotopic analysis. Paleoceanography, 19(3), n/a–n/a. doi:10.1029/2003pa000996 https://doi.org/10.1029/2003PA000996
Methods

Sambrook, J., Fritsch, E. F., & Maniatis, T. (1989). Molecular cloning: a laboratory manual (No. Ed. 2). Cold spring harbor laboratory press. https://isbnsearch.org/isbn/0879693096
Methods

Schumann, D., Ayranov, M., Stowasser, T., Gialanella, L., Di Leva, A., Romano, M., & Schuermann, D. (2013). Radiochemical separation of 7Be from the cooling water of the neutron spallation source SINQ at PSI. Radiochimca Acta, 101(8), 509-514.
Methods

Van Loon, J. C. (1985). Selected methods of trace metal analysis: biological and environmental samples. John Wiley and Sons.
Methods

Walter, H. J., Rutgers van der Loeff, M. M., & Hoeltzen, H. (1997). Enhanced scavenging of 231Pa relative to 230Th in the South Atlantic south of the Polar Front: Implications for the use of the 231Pa/230Th ratio as a paleoproductivity proxy. Earth and Planetary Science Letters, 149(1-4), 85–100. doi:10.1016/s0012-821x(97)00068-x https://doi.org/10.1016/S0012-821X(97)00068-X
Methods

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
Methods

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Parameters

Parameter	Description	Units
pH	pH	unitless
Th234_pcnt_activity_EPS_Si_plus	234Th exopolymeric substance extracted from diatoms cultured under Si-replete (f/2 medium) conditions	percent activity
Th234_pcnt_activity_EPS_Si_minus	234Th exopolymeric substance extracted from diatoms cultured under Si-depleted (f/2-Si medium) conditions	percent activity
Pa233_pcnt_activity_EPS_Si_plus	233Pa exopolymeric substance extracted from diatoms cultured under Si-replete (f/2 medium) conditions	percent activity
Pa233_pcnt_activity_EPS_Si_minus	233Pa exopolymeric substance extracted from diatoms cultured under Si-depleted (f/2-Si medium) conditions	percent activity
Be7_pcnt_activity_EPS_Si_plus	7Be exopolymeric substance extracted from diatoms cultured under Si-replete (f/2 medium) conditions	percent activity
Be7_pcnt_activity_EPS_Si_minus	7Be exopolymeric substance extracted from diatoms cultured under Si-depleted (f/2-Si medium) conditions	percent activity
Pb210_pcnt_activity_EPS_Si_plus	210Pb exopolymeric substance extracted from diatoms cultured under Si-replete (f/2 medium) conditions	percent activity
Pb210_pcnt_activity_EPS_Si_minus	210Pb exopolymeric substance extracted from diatoms cultured under Si-depleted (f/2-Si medium) conditions	percent activity
Po210_pcnt_activity_EPS_Si_plus	210Po exopolymeric substance extracted from diatoms cultured under Si-replete (f/2 medium) conditions	percent activity
Po210_pcnt_activity_EPS_Si_minus	210Po exopolymeric substance extracted from diatoms cultured under Si-depleted (f/2-Si medium) conditions	percent activity
pcnt_comp_Protein_EPS_Si_plus	Protein exopolymeric substance extracted from diatoms cultured under Si-replete (f/2 medium) conditions	percent composition
pcnt_comp_Protein_EPS_Si_minus	Protein exopolymeric substance extracted from diatoms cultured under Si-depleted (f/2-Si medium) conditions	percent composition
pcnt_comp_TCHO_EPS_Si_plus	Total carbohydrates exopolymeric substance extracted from diatoms cultured under Si-replete (f/2 medium) conditions	percent composition
pcnt_comp_TCHO_EPS_Si_minus	Total carbohydrates exopolymeric substance extracted from diatoms cultured under Si-depleted (f/2-Si medium) conditions	percent composition
pcnt_comp_Fe_EPS_Si_plus	Fe exopolymeric substance extracted from diatoms cultured under Si-replete (f/2 medium) conditions	percent composition
pcnt_comp_Fe_EPS_Si_minus	Fe exopolymeric substance extracted from diatoms cultured under Si-depleted (f/2-Si medium) conditions	percent composition

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Instruments

Dataset-specific Instrument Name	Pharmacia Biotech Multiphor II
Generic Instrument Name	Electrophoresis Chamber
Dataset-specific Description	For IEF (isoelectric focusing electrophoresis), a Pharmacia Biotech Multiphor II with a EPS3500 XL power supply was used.
Generic Instrument Description	General term for an apparatus used in clinical and research laboratories to separate charged colloidal particles (or molecules) of varying size through a medium by applying an electric field.

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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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