

Complexation Reactions Between Trace Metals and Specific Functional Groups in Natural Organic Matter from Estuarine Waters

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LONG-TERM GOALS

To gain a better understanding of the importance of colloidal macromolecules and microparticles in the cycling, speciation and bioavailability of trace elements in coastal waters. Special emphasis is placed on the role of acid polysaccharides, APS (major components in flocs, marine and freshwater “snow”, and biofilms) and reduced sulfur species (e.g., glutathione, phytochelatins and metallothionines) for trace metal complexation.

OBJECTIVES

- 1) Evaluation of isolation and purification procedures of natural thiols and APS for trace metal complexation experiments
- 2) Determination of concentrations and estuarine distributions of natural thiols and APS, and relationships to trace metal distributions.
- 3) Determination of conditional stability constants for the complexation of selected metals natural to biomolecules in Galveston Bay.
- 4) Determination of the physical forms and chemical structure of some of these biopolymers.

APPROACH

During the last year, PI Santschi, Postdoctoral Research Scientist Chin-Chang Hung, Assistant Research Scientist Laodong Guo, graduate student Kent Warnken worked on this project.

Approaches used in this project include:

- 1) Rigorous testing of cross-flow ultrafiltration (CFUF) methodology for the collection of colloidal macromolecular species of organic matter and trace metals.
- 2) Development of suitable methodologies for the measurement of thiols and polysaccharides (PS) in estuarine waters.

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- 3) Determination of thiol and PS distributions in riverine and coastal waters near Galveston in different molecular weight fractions, and relationship to those of trace metals.
- 4) Experimental determination of conditional stability constants for complexes between selected metals and biopolymers.
- 5) Investigation of the stability of colloids and separated biopolymers.

WORK COMPLETED

We have made good progress in many of the major objectives, especially in the following areas: 1) Publication of methodology improvements and results of the metal interactions with sulfides and thiols in the peer-reviewed literature. 2) Publication of the polysaccharide and uronic acid methodology and results of the distribution of acid polysaccharides in the peer-reviewed literature. 3) Improvement of the quantification of total APS in suspended matter using the alcian blue stain method. 4) Determination of the colloidal metal flux from the Trinity River and from Trinity Bay sediment pore waters in order to compare internal cycling to external river forcing in upper Galveston Bay.

RESULTS

Most of the Yr 2000/1 work on cross flow ultrafiltration, trace metal binding to colloids, relationships between total reduced sulfur concentration and metal complexing ligands, polysaccharide separation and distributions in Galveston Bay, and improvements in analytical detection of trace metals using ICP-MS has been carried through to publication (see publications list). Laboratory work was focused to improve methodology (APS, trace metals, colloidal trace metals) and to determine the importance of colloidal trace metals fluxes from sediments to the total flux balance in Trinity Bay, where the Trinity River enters Galveston Bay.

A) Results from the assessment of the cross-flow ultrafiltration technique for collecting environmental colloids

Cross-flow ultrafiltration (CFUF) can both overestimate and underestimate the amount of colloidal species (Guo et al., 2000, 2001c). Overestimation is produced by artifactual retention of small anionic species such as sulfate or low molecular weight metal-EDTA species by electrical interactions with the negatively charged membranes (Figure 1). Underestimation is produced by a de-facto molecular weight cut-off (MWCO) that is higher than the one stated by the manufacturer, due to malfunctioning of the cartridge, concentration dependence of the MWCO, or other reasons. It can be as bad as actual 50 kDa MWCO for a rated 1kDa MWCO membrane (Gustafsson et al., 1996). Only careful examination of the membrane cartridges and proper handling of samples can assure that problems have been minimized. Appropriate corrective actions include desalting by diafiltration after ultrafiltration, the use of high concentration factors, and extensive calibrations with standard molecules during every run (Guo et al., 2000, 2001c).

B) Relationship between total reduced sulfur concentration and metal complexing ligands

Partitioning of trace metals between the particulate ($\geq 0.45 \mu\text{m}$) and filter-passing ($< 0.45 \mu\text{m}$) fractions showed that Fe, Mn, and Pb had high exchangeable concentrations on particles, while Cu, Cd, Ni, and Zn were mostly present in the filter-passing fraction. In the filter-passing fraction, removal of the carrier phase trace metals (Fe as well as Mn) from the water was shown in lower salinity areas, while addition to the water column of other trace metals (Cd, Cu, Ni, Pb, and Zn) occurred in the mid-salinity region of Galveston Bay. The observed "particle concentration effect" of the partition coefficient (K_d) for Cu, i.e., the inverse dependence of K_d on suspended particulate matter concentration, disappears when the free ionic rather than the total concentrations of Cu in the filter-passing fraction is used for the calculation of K_d . This supports the theory that the presence of colloiddally bound trace metals in the filter-passing fraction is the main reason for the "particle concentration effect".

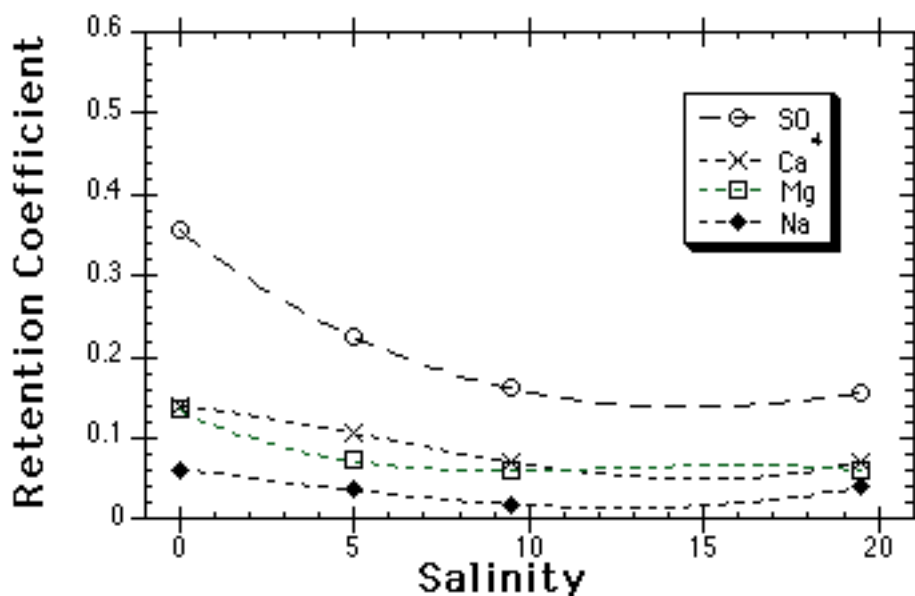


Figure 1. Decrease of retention coefficient ($R = 1 - P_c$) of both major anions and cations with increasing water salinity in a well-functioning CFUF system (Amicon). Anions are retained due to anion rejection of the negatively charged membrane, and cations due to electroneutrality. Species shown above include SO_4 , Ca, Mg, and Na (Guo et al., 2001c). Retention of these anions can be corrected for by subsequent diafiltration.

Organic complexation by reduced sulfur species was shown to be important for Cu, Ni, Zn, Cd and Pb in the filter-passing fraction in estuarine waters of Galveston Bay. The results of this work are in submission in Marine Chemistry (Tang et al., 2001).

C) Analytical improvements and determination of estuarine and oceanic distributions of polysaccharides

Acid polysaccharides are notoriously difficult to quantify, due to their strong tendency to form lactones irreproducibly upon acid hydrolysis, which moves them out of the analytical detection window. Therefore, methodology improvements were needed first, before any major study could be conducted.

We therefore adopted a multiple approach strategy:

1. We improved the methodology for the analysis of uronic acids, which are important components of the acid polysaccharide pool, by using ion exchange and lyophilization, in addition to initial esterization of the carboxylic acid group (Hung et al., 2001a).

2. We improved the quantitative aspect of the alcian blue staining method for the analysis of total acid polysaccharides (APS), a method that is common among oceanographers, by correcting for the amounts not retained by the filter using total polysaccharide rather than artifact-prone weight determinations. This improved standardization procedure is described in Hung et al. (2001b), where also its successful applications in a Gulf of Mexico program supported by NSF is reported. When using this improved methodology, more reasonable concentrations, and close correlations between APS and cyanobacteria abundance were observed (Figure 2), suggesting cyanobacteria as the source for APS.

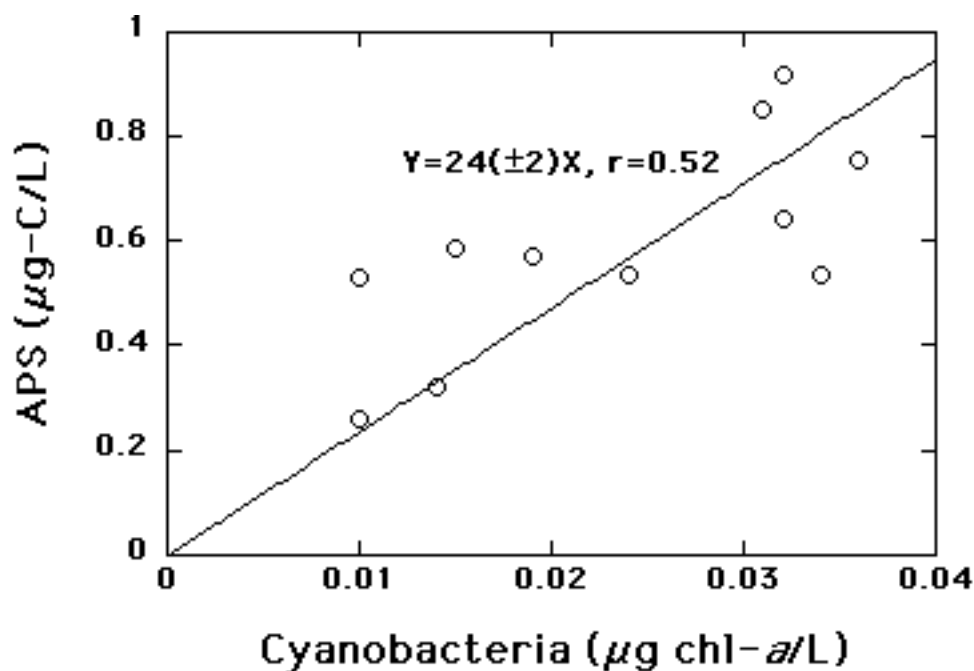


Figure 2. Linear relationship between APS and cyanobacteria abundance in small particles (0.7-10 μm) (Chin et al., 2001), suggesting that cyanobacteria are the source of APS.

3. We were able to obtain independent funding for the purchase of a GC-MS-MS (Polaris Q from Finnigan), with chemical ionization and solid and liquid sample introduction capability, to be used in FY2002 for the analysis of acid polysaccharides.

4. We also explored the possibility of adding a fluorescent tag to the terminal reducing sugar group of the polysaccharide chain, which will be useful for developing a gel electrophoresis/HPLC method using fluorescence detection in FY2002.

The concentrations of carbohydrates and total uronic acids in dissolved ($\leq 0.45\mu\text{m}$) and colloidal ($1\text{kDa} - 0.45\mu\text{m}$) phases were measured in estuarine waters of Galveston Bay, Texas, in order to study their role in heavy metal detoxification. Significant correlations of concentrations of polysaccharides (PCHO) and uronic acids to those of dissolved Cu ($\leq 0.45\mu\text{m}$) were found for the estuarine regions of Galveston Bay, which suggest that acid polysaccharides were produced in response to trace metal stressors. The results of this work have been published in Marine Chemistry (Hung et al., 2001c).

D) Improvements of trace metal analysis by ICP-MS for benthic flux determinations

Improvements in the analytical detection of trace metals in small sample volumes using ICP-MS, for applications to pore water analysis have been published (Warnken et al., 2000). In addition, total trace metal fluxes in Galveston Bay have also been published (Warnken et al., 2001) as well, while the detailed multi-year benthic flux chamber and pore water work on the importance of colloidal trace metals to benthic fluxes in Trinity Bay will be described in detail in the PhD dissertation of Kent Warnken, who will defend his PhD dissertation in October 2001.

IMPACT/APPLICATIONS

- 1) Improvements in APS detection methodology supported by this program lead to insights into important synergistic relationships between metals (such as Cu or Th) and acid polysaccharides produced by cyanobacteria, in another program funded by NSF.
- 2) The PhD dissertation of Kent Warnken on benthic trace metal fluxes will be defended in Oct. 2001.
- 3) The fact that many publications resulting from this research are frequently cited in the literature suggests that the results from this research are heavily utilized by other researchers in the field.

TRANSITIONS

Our analytical methodology, hardware and software have benefited a number of studies listed below.

RELATED PROJECTS

This project benefited from other programs directed by Peter H. Santschi, which investigate environmental colloids. They include: 1) Kaiser-Hill/Dept. of Energy, "Actinide Migration Studies at the Rocky Flats Environmental Technology Site", where we were able to show that actinides in surface waters are associated with macromolecular organic colloids (Santschi et al., 2001). 2) Texas Advanced Research Program, "Reconstruction of Terrestrial ^{129}I Inputs into Marine Environments", where we showed that ^{129}I , an anthropogenically produced and long-lived radionuclide of iodine, is mainly associated with macromolecular organic colloids in the Mississippi River (Oktay et al., 2001); 3) Texas Seagrant. "Bioavailability of colloid-associated metals to estuarine bivalves", where we showed that estuarine bivalves are able to take up colloiddally bound trace metals (Guo et al., 2001a); 4)

National Science Foundation, " Interactions of Th(IV) with Organic Compound Classes of Marine Organic Matter", where we demonstrated significant relationships between $^{234}\text{Th}/\text{OC}$ Ratios, acid polysaccharide and cyanobacteria abundance in the Gulf of Mexico (Guo et al., 2001b; Hung et al., 2001b). In the laboratory, we were able to isolate an acid polysaccharide of 12.5 kDa molecular weight with strong trace metal and ^{234}Th affinity (Quigley et al., 2001). International collaborations with leaders in the field, Drs. J. Buffle and K. Wilkinson, Dept. of Analytical Chemistry, University of Geneva, Switzerland (<http://www.unige.ch/cabe>), and participation in PhD dissertation committees of two chemistry students, greatly helped in our environmental colloid work.

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PUBLICATIONS 2000-2001, supported, in parts, by ONR.

A) Publications in Peer-Reviewed Journals:

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