Characterization of the Structural and Chemical Properties of Copper Chelators in Seawater

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LONG-TERM GOALS

The long-term goal is to obtain a comprehensive understanding of copper chemistry and bioavailability in seawater, at the molecular level. We are particularly interested in the relationship between water chemistry and biological effects of copper. This information can be used for accurate assessments of the impacts of Cu introduced to harbors by human activities. Such information may also be useful in the development of accurate and economical strategies to detect and remove Cu and other contaminants from waste. Results can be used by dischargers, like the US Navy, and regulators, including the EPA and local agencies, to make informed decisions about managing Cu inputs into harbors and other receiving waters. A major long-term goal is to establish linkage between basic research in this area and the regulatory and remediation communities.

OBJECTIVES

The primary objectives of this project relate to naturally occuring Cu binding ligands. Our work, and that of others, shows that these ligands control the variability in Cu bioavailability in many coastal waters. We seek to learn more about the chemical properties of these poorly characterized substances. Current titration methodologies provide information about binding constants and concentrations only. Structural information is necessary to validate hypotheses about sources and sinks (with a view to modeling variability) and to identify compounds with unique properties of relevance to the Navy.

We are also interested in new ways to characterize Cu binding in coastal seawater and discharge waters using rapid, real-time methodologies that can be used to a wide range of investigators and are not prone to interferences from surfactants in the way that existing electrochemical methods are.

We are also interested in linking the Harbor Processes Community with the regulatory community directly in ways that may provide more rational approaches to regulating Cu in coastal waters.

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14. ABSTRACT

The long-term goal is to obtain a comprehensive understanding of copper chemistry and bioavailability in seawater, at the molecular level. We are particularly interested in the relationship between water chemistry and biological effects of copper. This information can be used for accurate assessments of the impacts of Cu introduced to harbors by human activities. Such information may also be useful in the development of accurate and economical strategies to detect and remove Cu and other contaminants from waste. Results can be used by dischargers, like the US Navy, and regulators, including the EPA and local agencies, to make informed decisions about managing Cu inputs into harbors and other receiving waters. A major long-term goal is to establish linkage between basic research in this area and the regulatory and remediation communities.

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APPROACH

Molecular-level studies focus on chelators produced by ubiquitous marine phytoplankton and bacteria, rather than chelators actually in the water column, because they can be produced at much higher concentrations. Our earlier Harbor Processes work showed that some organisms might be important sources, such as the marine cyanobacterium *Synechococcus*. Cu stressed *Synechococcus* cultures produce a chelator with binding characteristics similar to the strongest ligands found in the water column. We hypothesized that this ligand is produced as a detoxification mechanism for Cu (Moffett and Brand, 1996). We are approaching this using a liquid chromatography electrospray mass spectrometric (LC-ESMS) methodology.

We are also using two approaches to real-time speciation measurements. We are working with Richard Thompson (U. Maryland) who has 6.2 support to develop a cupric ion fiber optic biosensor to intercalibrate with existing methods over a range of free Cu concentrations. We are also working to develop our own methodology, based on a colorimetric ligand for Cu(II) and long pathlength spectrophotometric detection.

WORK COMPLETED

Last year, we presented substantial evidence that Cu is coordinated by reduced S ligands in cyanobacterial cultures. This was consistent with Peter Santschi's work on glutathione and other thiols in coastal waters. This year, we developed and optimized an LC-ESMS method based on the derivatizing agent monobromobimane. First we optimized the LC protocols to efficiently derivatize model ligands and separate them from naturally occurring thiols present in the water column and cultures. Secondly, we developed protocols to generate useful mass spectra from the derivatized thiols.

We have developed a protocol to determine Cu(II) colorimetrically in seawater and showed that the method could be modified to characterize Cu(II) speciation.

We intercompared the Thompson biosensor with cathodic stripping voltammetry in July 2001 in Woods Hole MA. The sensor was positioned for 24h in a tidal channel where free Cu^{2+} varies by 1000x over a tidal cycle due to flushing of a contaminated basin. We also worked with Thompson in the months preceding the intercomparison to help him calibrate his system with model ligands.

RESULTS

Initially, we had difficulty obtaining consistent positive ion spectra, but eventually established workable conditions. We identified glutathione unequivocally (Figure 1) but established that it was a minor contributor to the thiol peak detected electrochemically in the cultures.

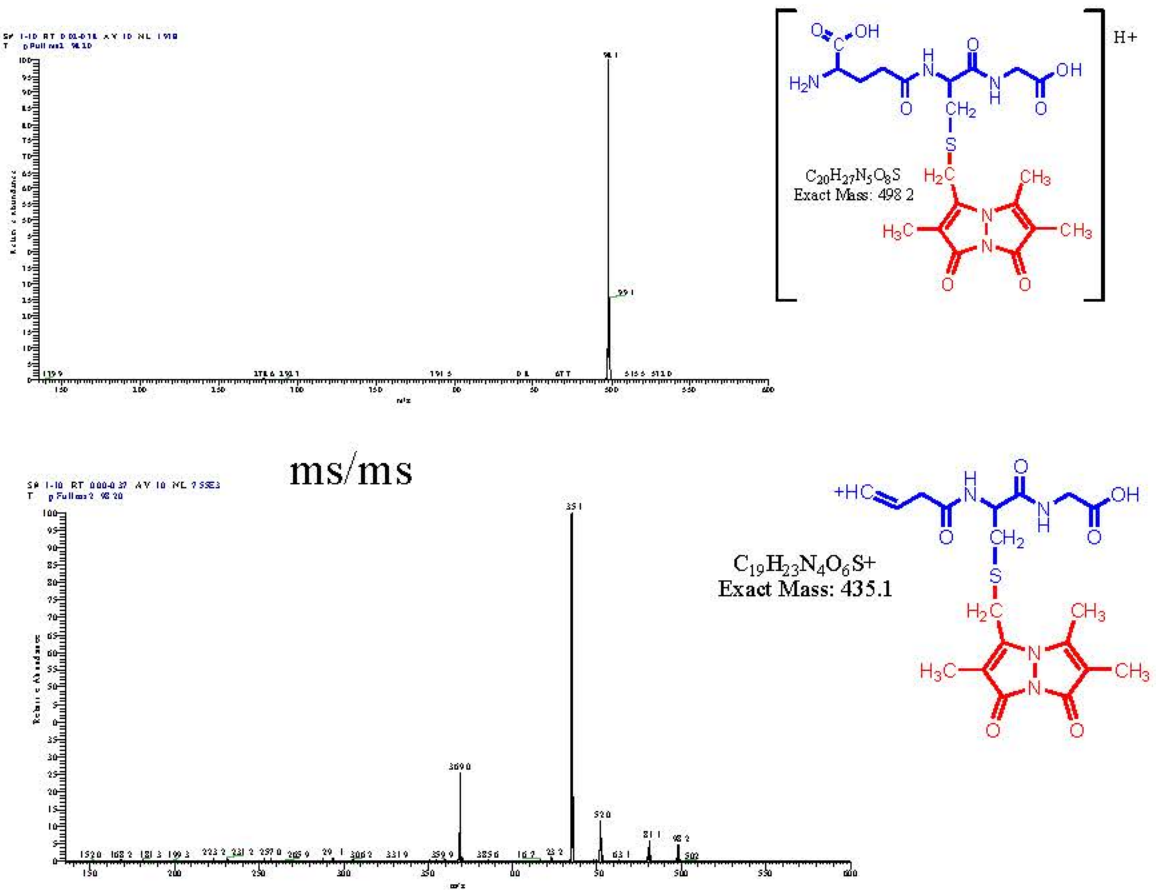

Figure 1. Top: Mass spectrum showing parent ion of glutathione monobromobimane adduct (structure top right). Bottom: Spectrum when just the parent ion is trapped and broken up in the absence of other compounds (structure of dominant product at bottom right).

However, another compound, that appears to be doubly derivatized (and thus contains two thiol groups) was detected. Tentatively we identified this compound as the cysteine-cysteine dipeptide. Such a compound would be a strong Cu chelator, but is also readily oxidized to a disulfide. Interestingly, we only detect this compound when we de-metallate the ligand (by acidification to pH 2) prior to derivatization, suggesting that it is strongly bound to copper at pH 8, blocking derivatization. Further work is ongoing to establish the conditions under which this compound is produced. Interestingly, Cu stressed cultures produce a substantial concentration of hydrogen sulfide as well (detectable by smell). While this has been reported before by Cutter and coworkers, the relationship between H_2S and thiols is unknown.

Last year we developed LC-ESMS protocols for fluorescamine, a derivatizing agent for primary amines that is widely used for peptide analysis. This reagent derivatizes peptide thiols, like glutathione or cysteine- cysteine, providing additional structural information.. In cultures derivatization produces several peaks. However, cultures containing strong Cu ligands have a single peak that is much larger than the rest. ESMS analysis suggests that this is a discrete compound. We are actively working on further fragmentation of the parent ion to determine whether this peak is a thiol peptide.

The intercomparison showed good agreement with CSV and the biosensor at the low end of the concentration range (pM free Cu^{2+}), but CSV yielded consistently higher results at the high Cu samples. This could be due to a saturation effect on the biosensor, but may also be due to surfactant interferences with CSV for certain samples.

Cuprizone is an effective chelator of Cu(II) in seawater and has an extinction coefficient of approximately 20,000. This should yield a detection limit in the nanomolar range in seawater using the Ocean Optics long pathlength spectrophotometer. Ligand competition experiments using DTPA suggest that the binding affinity of cuprizone is in a useful range for natural systems.

IMPACT/APPLICATIONS

Successful characterization of this material could lead to new insight into the sources and chemistry of Cu ligands in seawater. Potential applications could arise if we identify a new class of chelators selective for Cu that could be used in remediation.

Moffett organized a workshop that was held in Woods Hole in July 2001 to discuss how new regulatory criteria for copper that take into account organic complexation might be adopted by the EPA. The workshop brought together several PI s from the Harbor Processes program with toxicologists and environmental engineers who have long been the principal link between the academic community and the EPA. Moffett's intention was to strengthen these ties and involve Harbor Processes Investigators in activities where they can influence the regulatory process directly.

The Thompson biosensor could have a large impact on the ease of copper speciation measurements. Calibration against the existing methodologies by our group could hasten its acceptance by the marine community.

TRANSITIONS

None recognized at this time.

RELATED PROJECTS

Moffett is collaborating with Beth Ahner (Cornell) who is working on strategies to preconcentrate thiols from natural waters and isolate them by HPLC. Moffett is also supported by NSF to develop derivatization strategies for marine siderophores.

Moffett also works with Brian Palenik (Scripps) on a project to study the relationship between Cu chemistry in seawater and the production of a highly Cu specific cell-surface binding protein by marine diatoms. Palenik has developed an antibody to this protein that may be an excellent in situ indicator of metal stress.

PUBLICATIONS

AASERT Awards tied to this project supported those marked with an asterisk.

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*Barbeau K., E.B. Kujawinski and J. W. Moffett. Remineralization and recycling of iron, thorium and organic carbon by heterotrophic marine protists in culture. Submitted to *Aquatic Microbial Ecology.*

*Kujawinski, Elizabeth B., John W. Farrington[2], and James W. Moffett. Evidence for grazing-mediated production of surface-active material by marine protozoa. Submitted to *Marine Chemistry.*

* Kujawinski, E.B., J.W. Farrington and J.W. Moffett. Protozoan grazers produce organic matter with an exceptionally high affinity for PCBs. In Press. *Environmental Science and Technology.*