1 Exploring the ability of cations to facilitate binding
2 between inorganic oxyanions and humic acid

3 *David P. Martin*, Jennifer M. Seiter, Brandon J. Lafferty, and Anthony J. Bednar*

4 U.S. Army Engineer Research and Development Center, Vicksburg, MS 39180, US

Abstract

The mobility and fate of inorganic oxyanions in the environment can be greatly influenced by interactions with natural organic matter (NOM). There is increasing evidence that this interaction between two anionic species is facilitated by cationic bridges, but detailed mechanistic studies into this system are challenging due to the heterogeneous nature of NOM. This work examines the ability of cations (Fe^{III} , Cr^{III} , Al^{III} , or Ga^{III}) to form ternary complexes with Suwannee River humic acid (SRHA) and the oxyanions of As (As^{III} and As^{V}) and Se (Se^{IV} and Se^{VI}). Complexes were characterized by separating SRHA from unbound species using size exclusion chromatography coupled to ICP-MS to determine its metal content. Unlike Cr^{III} and Fe^{III} , the post-transition metal ions Al^{III} and Ga^{III} proved ineffective at forming ternary complexes with any of the oxyanions, although minor complexation was observed with Ga^{III} , suggesting that electrostatic interactions are not the primary driving force behind the stabilization of these ternary complexes. The results also show differences in the behavior of Fe^{III} and Cr^{III} that may indicate that the two cations stabilize the ternary complexes by different mechanisms.

Keywords: Humic acid; SEC-ICP-MS; ternary complexes; arsenic; selenium

1. Introduction

Inorganic contamination is of great concern due to both the high toxicity of this class of materials as well as their environmental persistence.¹ Inorganic contamination can arise from a variety of anthropogenic sources including mining, farming, and industrial processes and can pose a significant threat to human health and the environment. Accurate predictions of the fate and transport of inorganic species in the environment are critical to both remediation and risk assessment; the behavior of inorganic ions in the environment, however, is dependent on a number of interdependent processes including dissolution/precipitation, oxidation/reduction, biotransformation, and sorption to components of the environmental matrix that can be difficult to model.²

Complexation of inorganic ions by natural organic matter (NOM) is one factor that can have a significant impact on their speciation and transport in soil and water systems. Since NOM contains a large number of electron donor groups (e.g. CO_2H , OH , NH_2),³ it can readily bind cationic metal species and these interactions have been relatively well-studied.⁴⁻¹¹ Despite being an anionic species itself, NOM has also been observed to bind inorganic oxyanions, such as those of arsenic (As^{V} as AsO_4^{3-} and As^{III} as AsO_3^{3-})¹²⁻¹⁵ and selenium (Se^{VI} as SeO_4^{2-} and Se^{IV} as SeO_3^{2-}).¹⁶⁻²⁰ Recently, evidence has been accumulating that the interactions between the two anionic species are mediated by bridging cations, particularly Fe^{III} .²¹⁻²⁴ This binding mechanism is not limited to inorganic oxyanions; similar ternary complexes have been shown to form between NOM and organic tetracyclines.²⁵

The objective of this study was to gain insight into the coordination chemistry that governs the formation of ternary complexes between NOM, metal cations, and oxyanions. The ability of trivalent cations to facilitate the formation of ternary complexes between Suwannee River humic

acid (SRHA) and the oxyanions of As and Se was determined by using size exclusion chromatography to separate the NOM from ions free in solution interfaced to ICP-MS for element-specific analysis. The ability of SRHA loaded with different metals (Fe^{III} , Cr^{III} , Al^{III} , and Ga^{III} , which carry a similar charge but vary in their chemical properties) to bind inorganic oxyanions can provide insight into how these cations facilitate the formation of ternary complexes between NOM and oxyanions; this information can provide a valuable complement to direct spectroscopic methods to more accurately describe the binding mechanisms governing these systems.

2. Materials and Methods

2.1. Materials

Chemicals (reagent grade or higher purity) were obtained from commercial sources and used without further purification. Suwannee River humic acid (1.04% ash content; a more complete description of the material can be found in the Supplementary Information) was obtained from the International Humic Substances Society and used without further purification. All aqueous solutions were prepared using deionized ($18.2 \text{ M}\Omega\cdot\text{cm}$) water.

2.2. Sample Preparation

Stock solutions of humic acid (1 g/L) were prepared in HPLC mobile phase while those for metal salts (100 mg/L) were prepared in water. The salts used for this study were FeCl_3 , $\text{Cr}(\text{NO}_3)_3$, $\text{AlK}(\text{SO}_4)_2$, $\text{Ga}(\text{NO}_3)_3$, Na_2HAsO_4 , NaAsO_2 , Na_2SeO_4 , and Na_2SeO_3 . Samples (1 mL) were prepared by the addition of metal stock solutions into mobile phase containing 25 mg/L humic acid and allowed to incubate overnight. Samples were centrifuged (15 min at 10,000 RPM) prior to analysis to remove any precipitated species. Although precipitate was visible in the Fe^{III} and Al^{III} trials, analysis of the total organic carbon in the supernatant indicated that the

concentration of SRHA had not decreased significantly (<5%); separate analysis showed that roughly 50% of the Fe^{III} and all of the other metals remained in solution following the incubation period. The final nominal cation concentrations, which correspond to roughly 0.3 equivalents relative to the carboxylic acid content of the SRHA, were 1 mg/L for Fe^{III} , Cr^{III} , and Ga^{III} and 0.5 mg/L for Al^{III} . The final oxyanion concentrations were 0.25 mg/L for As^{V} and 1 mg/L for As^{III} , Se^{VI} , and Se^{IV} . No significant differences were observed in the data when the SRHA was incubated with cations to allow formation of binary complexes prior to the addition of oxyanions.

2.3. Size Exclusion Chromatography

Separation of a 50 μL injection was performed using an Agilent 1100 High Performance Liquid Chromatography (HPLC) system with a Shodex OH pak SB803 HQ column (analytical range 1-100 kDa; exclusion limit 100 kDa) running a mobile phase of 10 mM sodium acetate (pH 7.0, adjusted with HNO_3) with 1% methanol and a flow rate of 0.75 mL/min. Polystyrenesulfonate standards were used to correlate elution time and molecular weight (Figure S1). Real-time metals detection of the eluent was obtained by interfacing the HPLC system with a Perkin Elmer Elan DRC II ICP-MS using a MiraMist pneumatic nebulizer. Since background signal was not a concern, the ICP-MS was operated in standard mode with the plasma operated at 1300 W and a nebulizer flow rate of 0.9 mL/min. Data were background-subtracted using Origin software.

3. Results and Discussion

3.1. Binary Complex Formation

Although the complexes between SRHA and the metal cations used in this study have been previously described in the literature,⁴⁻¹¹ initial experiments were performed to verify their

formation under the current conditions. SRHA was incubated overnight with increasing concentrations of Fe^{III} , Cr^{III} , Al^{III} , and Ga^{III} and centrifuged prior to analysis to remove any precipitated species. Size exclusion chromatography was used to separate the SRHA from free metal ions in solution and the metal content of the eluent monitored by ICP-MS. Metal ions that coelute with the SRHA (as indicated by its UV absorbance) are assumed to be bound by it; control experiments were performed in the absence of SRHA to verify no colloidal species that may elute at a time similar to that of SRHA were formed. It should be noted that the Fe content of the as-received SRHA was found to be roughly 50 nmol/mg (as determined by direct analysis by Inductively Coupled Plasma Atomic Emission Spectroscopy) and SEC-ICP-MS analysis shows that this Fe is complexed with the SRHA (Figure S2). When the SRHA is incubated with increasing concentrations of the cations, its secondary structure begins to change and, if the cation concentration is sufficiently high, it precipitates from solution (Figure S3); all subsequent experiments were performed at concentrations that maximized the cation loading but did not result in significant changes to the SRHA SE chromatogram. In the case of Fe, the amount of metal added to the solution is roughly 40-fold higher than the native Fe content of the as-received SRHA. The metal:SRHA ratio in the purified fractions under these conditions were similar to the metal binding capacities previously reported in the literature.²⁶

3.2. Ternary Complexes of As

Ternary complexes between cation-loaded SRHA and oxyanions were detected by SEC-ICP-MS as described above for the binary complexes (Figure 1). When As^{V} is incubated with the cations in the absence of SRHA (labeled ‘ As^{V} Control’ in Figure 1A but omitted from the other three panels for clarity), no As signal was observed with an elution time similar to that of SRHA. While the Fe^{III} -mediated ternary complex has been characterized previously in the literature, the

ability of other metals to facilitate this molecular interaction is far less studied. When As^V was incubated with SRHA and either Fe^{III} (Figure 1A) or Cr^{III} (Figure 1B), a significant As signal was observed during the elution of SRHA, indicating the formation of ternary complexes. Furthermore, the ICP-MS signals for Fe and Cr coincide with those of As in these experiments. It should be noted that despite containing some native Fe, no interaction was observed when SRHA was incubated with As^V in the absence of added cations (Figure S4). While the Cr^{III} appears to be relatively evenly distributed throughout the SRHA, from comparison of the ICP-MS and UV chromatograms, the Fe^{III} is preferentially bound to the higher molecular weight fractions (those with shorter retention times); this is consistent with previous studies using SEC to analyze humic-metal binding.²⁷ In addition, the As^V appears to interact preferentially with the Fe^{III} bound to the higher molecular weight fractions of the SRHA whereas the distribution is far more even for the Cr^{III}-mediated complex. This could potentially indicate that the two cations facilitate the formation of As^V ternary complexes through different mechanisms. X-ray absorption near edge structure (XANES) of the SEC-purified complexes (Figure S5) shows that the oxidation state of the As does not change upon binding. It should be noted that a small shoulder is present in the As XANES of the Cr^{III}-mediated complex that corresponds to the presence of <5% reduced As species. The most likely explanation is that some of the As^V in the sample was reduced either by the Cr^{III} or by the X-ray beam itself.

In the case of Al^{III}-loaded SRHA (Figures 1C) there is no evidence for the formation of a complex with SRHA and As^V, suggesting that electrostatic attraction alone is not sufficient for a cation to facilitate ternary complex formation. The ionic radii of the cations may also play a role, but Ga^{III}, which is more similar in size to the transition metal ions, does not facilitate the

formation of a significant amount of the As^{V} ternary complex either (Figure 1D) although a very small amount of As does coelute with the Ga^{III} -loaded SRHA (Figure S6).

Figure 1. Representative size exclusion chromatograms of ICP-MS signal (solid lines) and UV absorbance (dotted lines) during separation of SRHA incubated with As^{V} and cations (A: Fe^{III} , B: Cr^{III} , C: Al^{III} , D: Ga^{III}). Although all four cations form binary complexes with SRHA, only Fe^{III} and Cr^{III} facilitate the formation of ternary complexes with SRHA and As^{V} . The control experiment with no SRHA included in the incubation, labeled ‘ As^{V} Control’, is shown in panel A; the results were similar for the other three metals but omitted for clarity. Interactions between As^{V} and the resin result in a longer retention time than other low molecular weight species.

To further probe the determinants of ternary complex formation, the same experiment was performed with As^{III} , which differs from As^{V} in both oxyanion geometry and pK_{a} .²⁸ The data shows the formation of Fe^{III} -mediated complexes between As^{III} and SRHA (Figure 2), which has been demonstrated previously.¹⁴ The other cations, most surprisingly Cr^{III} , did not form ternary complexes with As^{III} and SRHA under these experimental conditions (Figure S6). Much like As^{V} , the As^{III} is associated with the higher molecular weight fraction of the Fe^{III} -loaded SRHA

and coelutes with the Fe ICP-MS signal. X-ray absorption near edge structure (XANES) of the purified ternary complex verifies that As does in fact bind as As^{III} rather than binding after oxidation to As^V (Figure S5C). The lack of SRHA-Cr^{III}-As^{III} ternary complexes further demonstrates that the properties of the bridging metal ion play a significant role in the formation of these complexes and provides further support for the notion that the two metals act through different mechanisms.

Figure 2. Representative size exclusion chromatograms of ICP-MS signal (solid lines) and UV absorbance (dotted line) during separation of SRHA incubated with Fe^{III} and As^{III}. Unbound As^{III} elutes at >15 min (see Figure S7).

3.3. Ternary Complexes of Se

The contrasting behavior of As^{III} and As^V described above for Fe^{III}- and Cr^{III}-loaded SRHA suggests that the properties of the oxyanion have a significant influence on ternary complex formation; to further examine this effect, the ability of cation-loaded SRHA to form complexes with the oxyanions of selenium was studied. Consistent with previous reports that showed no interaction between Se^{VI} and NOM,¹⁸ no ternary complexes were observed with Se^{VI} and SRHA in the presence of any of the cations studied (Figure S8). In the case of Se^{IV}, however, ternary

complexes were formed with both Fe^{III} - and Cr^{III} -loaded SRHA (Figure 3). Unlike the ternary complexes between As and Fe^{III} -loaded SRHA, in which the oxyanion appeared to only be associated with the higher molecular weight fractions, the Se is relatively evenly distributed throughout the SRHA. The peak corresponding to the Cr^{III} -mediated complex (Figure 3B) is small and may indicate relatively weak binding, although the relatively low abundance of the ^{82}Se isotope used for detection is also a factor in the visual representation.

Figure 3. Representative size exclusion chromatograms of ICP-MS signal (solid lines) and UV absorbance (dotted lines) during separation of SRHA incubated with Se^{IV} and either Fe^{III} (A) or Cr^{III} (B).

4. Conclusions

Taken together, these results indicate that a variety of forces govern the formation of cation-mediated ternary complexes between NOM and inorganic oxyanions. Although Cr^{III} - or Ga^{III} -mediated ternary complexes may have limited environmental relevance, studying them can provide a valuable complement to mechanistic studies, such as those using EXAFS and other spectroscopy techniques, to gain fundamental insight into these complex systems. Charge neutralization alone does not appear to be sufficient for the cationic bridge; the behavior of Fe^{III} and Cr^{III} relative to the post-transition metal ions Al^{III} and Ga^{III} suggest that the properties of the transition metals are critical to stabilization of the ternary complexes with NOM and inorganic oxyanions. Furthermore, the divergent behavior of Fe^{III} and Cr^{III} suggest that the two cations may

form ternary complexes through different mechanisms. Understanding this fundamental coordination chemistry is critical to developing a robust model for determining the fate and transport of metal oxyanions in the environment.

Author Information

***Corresponding author e-mail: david.p.martin@usace.army.mil; address: U.S. Army Engineer Research and Development Center, Environmental Laboratory EP-C, 3909 Halls Ferry Rd., Vicksburg, MS 39180**

Acknowledgments

The use of trade, product, or firm names in this report is for descriptive purposes only and does not imply endorsement by the U.S. Government. The tests described and the resulting data presented herein, unless otherwise noted, were obtained from research conducted under the Environmental Quality Technology Program of the United States Army Corps of Engineers by the USAERDC. Portions of this work were performed at GeoSoilEnviroCARS (The University of Chicago, Sector 13), Advanced Photon Source (APS), Argonne National Laboratory. GeoSoilEnviroCARS is supported by the National Science Foundation - Earth Sciences (EAR-1128799) and Department of Energy- GeoSciences (DE-FG02-94ER14466). This research used resources of the Advanced Photon Source, a U.S. Department of Energy (DOE) Office of Science User Facility operated for the DOE Office of Science by Argonne National Laboratory under Contract No. DE-AC02-06CH11357. Permission was granted by the Chief of Engineers to publish this information. The findings of this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents. The authors also thank Drs. Dale Rosado and Afrachanna Butler of the USACE for their editorial comments.

- (1) Wuana, R. A.; Okieimen, F. E. Heavy Metals in Contaminated Soils: A Review of Sources, Chemistry, Risks and Best Available Strategies for Remediation *ISRN Ecol.* **2011**, *2011*, 20
- (2) Violante, A.; Cozzolino, V.; Perelomov, L.; Caporale, A. G.; Pigna, M. Mobility and Bioavailability of Heavy Metals and Metalloids in Soil Environments *J. Soil Sci. Plant Nutr.* **2010**, *10*, 268-292
- (3) Chen, J.; Gu, B.; LeBoeuf, E. J.; Pan, H.; Dai, S. Spectroscopic characterization of the structural and functional properties of natural organic matter fractions *Chemosphere* **2002**, *48*, 59-68
- (4) Marx, G.; Heumann, G. K. Mass spectrometric investigations of the kinetic stability of chromium and copper complexes with humic substances by isotope-labelling experiments *Fresenius J. Anal. Chem.* **1999**, *364*, 489-494
- (5) Tipping, E.; Rey-Castro, C.; Bryan, S. E.; Hamilton-Taylor, J. Al(III) and Fe(III) Binding by Humic Substances in Freshwaters, and Implications for Trace Metal Speciation *Geochim. Cosmochim. Acta* **2002**, *66*, 3211-3224
- (6) Hagvall, K.; Persson, P.; Karlsson, T. Spectroscopic characterization of the coordination chemistry and hydrolysis of gallium(III) in the presence of aquatic organic matter *Geochim. Cosmochim. Acta* **2014**, *146*, 76-89
- (7) Piana, M. J.; Zahir, K. O. Investigation of Metal Ions Binding of Humic Substances Using Fluorescence Emission and Synchronous-Scan Spectroscopy *J. Environ. Sci. Health B* **2000**, *35*, 87-102
- (8) Pandey, A. K.; Pandey, S. D.; Misra, V. Stability Constants of Metal-Humic Acid Complexes and its Role in Environmental Detoxification *Ecotoxicol. Environ. Saf.* **2000**, *47*, 195-200
- (9) Weng, L.; Temminghoff, E. J. M.; Lofts, S.; Tipping, E.; Van Riemsdijk, W. H. Complexation with Dissolved Organic Matter and Solubility Control of Heavy Metals in a Sandy Soil *Environ. Sci. Technol.* **2002**, *36*, 4804-4810
- (10) Gustafsson, J. P.; Persson, I.; Oromieh, A. G.; van Schaik, J. W. J.; Sjostedt, C.; Kleja, D. B. Chromium(III) Complexation to Natural Organic Matter: Mechanisms and Modeling *Environ. Sci. Technol.* **2014**, *48*, 1753-1761
- (11) Sutheimer, S. H.; Cabaniss, S. E. Aluminum Binding to Humic Substances Determined by High Performance Cation Exchange Chromatography *Geochim. Cosmochim. Acta* **1997**, *61*, 1-9
- (12) Buschmann, J.; Kappeler, A.; Lindauer, U.; Kistler, D.; Berg, M.; Sigg, L. Arsenite and Arsenate Binding to Dissolved Humic Acids: Influence of pH, Type of Humic Acid, and Aluminum *Environ. Sci. Technol.* **2006**, *40*, 6015-6020
- (13) Anawar, H. M.; Akai, J.; Komaki, K.; Terao, H.; Yoshioka, T.; Ishizuka, T.; Safiullah, S.; Kato, K. Geochemical occurrence of arsenic in groundwater of Bangladesh: sources and mobilization processes *J. Geochem. Explor.* **2003**, *77*, 109-131
- (14) Liu, G.; Fernandez, A.; Cai, Y. Complexation of Arsenite with Humic Acid in the Presence of Ferric Iron *Environ. Sci. Technol.* **2011**, *45*, 3210-3216
- (15) Ritter, K.; Aiken, G. R.; Ranville, J. F.; Bauer, M.; Macalady, D. L. Evidence for the Aquatic Binding of Arsenate by Natural Organic Matter-Suspended Fe(III) *Environ. Sci. Technol.* **2006**, *40*, 5380-5387

- (16) Kamei-Ishikawa, N.; Nakamaru, Y.; Tagami, K.; Uchida, S. Sorption Behavior of Selenium on Humic Acid Under Increasing Selenium Concentration or Increasing Solid/Liquid Ratio *J. Environ. Radioactiv.* **2008**, *99*, 993-1002
- (17) Gustafsson, J. P.; Johnsson, L. The association between selenium and humic substances in forested ecosystems—laboratory evidence *Appl. Organomet. Chem.* **1994**, *8*, 141-147
- (18) Bruggeman, C.; Maes, A.; Vancluysen, J. The Interaction of Dissolved Boom Clay and Gorleben Humic Substances with Selenium Oxyanions (Selenite and Selenate) *Appl. Geochem.* **2007**, *22*, 1371-1379
- (19) Kamei-Ishikawa, N.; Tagami, K.; Uchida, S. Sorption Kinetics of Selenium on Humic Acid *J. Radioanal. Nucl. Chem.* **2007**, *274*, 555-561
- (20) Johnsson, L.; Gustafsson, J. P. The Association between Selenium and Humic Substances in Forested Ecosystems-Laboratory Evidence *Appl. Organomet. Chem.* **1994**, *8*, 141-147
- (21) Sharma, P.; Ofner, J.; Kappler, A. Formation of Binary and Ternary Colloids and Dissolved Complexes of Organic Matter, Fe, and As *Environ. Sci. Technol.* **2010**, *44*, 4479-4485
- (22) Hoffmann, M.; Mikutta, C.; Kretzschmar, R. Arsenite Binding to Natural Organic Matter: Spectroscopic Evidence for Ligand Exchange and Ternary Complex Formation *Environ. Sci. Technol.* **2013**, *47*, 12165-12173
- (23) Mikutta, C.; Kretzschmar, R. Spectroscopic Evidence for Ternary Complex Formation Between Arsenate and Ferric Iron Complexes of Humic Substances *Environ. Sci. Technol.* **2011**, *45*, 9550-9557
- (24) Sundman, A.; Karlsson, T.; Sjöberg, S.; Persson, P. Complexation and Precipitation Reactions in the Ternary As(V)–Fe(III)–OM (Organic Matter) System *Geochim. Cosmochim. Acta* **2014**, *145*, 297-314
- (25) MacKay, A. A.; Canterbury, B. Oxytetracycline Sorption to Organic Matter by Metal-Bridging *J. Environ. Qual.* **2005**, *34*, 1964-1971
- (26) Čežíková, J.; Kozler, J.; Madronová, L.; Novák, J. r.; Janoš, P. Humic acids from coals of the North-Bohemian coal field: II. Metal-binding capacity under static conditions *React. Funct. Polym.* **2001**, *47*, 111-118
- (27) Bolea, E.; Gorriz, M. P.; Bouby, M.; Laborda, F.; Castillo, J. R.; Geckeis, H. Multielement characterization of metal-humic substances complexation by size exclusion chromatography, asymmetrical flow field-flow fractionation, ultrafiltration and inductively coupled plasma-mass spectrometry detection: A comparative approach *Journal of Chromatography A* **2006**, *1129*, 236-246
- (28) Bissen, M.; Frimmel, F. H. Arsenic - A Review. Part I. Occurrence, Toxicity, Speciation, Mobility *Acta Hydrochim. Hydrobiol.* **2003**, *31*, 9-18

367

Graphical Abstract

368