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MOLECULAR INTERACTIONS OF TOXIC METALS

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Project Description

This project examines the common aspect that unites all toxic metal pathologies: directly disruptive chemical interactions that functionally impair important biomolecules. The molecular interactions that occur between selenium and mercury and between nickel subsulfide and deoxyribonucleic acid (DNA) are responsible for their pathophysiological effects. Selenium availability diminishes methylmercury bioaccumulation in fish and diminishes methylmercury toxicity in all species studied. Both of these effects seem to occur as a result of mercury selenide formation during normal selenium metabolism within animal cells. In the case of the nickel subsulfide–DNA interaction, a unique and unexpected molecular mechanism of DNA adduct formation may be responsible for its damaging effects. This project examines the molecular reactions responsible for the physiological and ecological effects of these toxic metals.

Goals

The overall goal of this project is to increase the understanding of toxic metals and the biochemical mechanisms of their disruptions of cell physiology that appear to be responsible for their pathological effects. The goal of the first two studies is to examine the biogeochemistry of Hg–Se interactions and assess the effects of endogenous selenium on Hg bioaccumulation and retention. The third study will examine the nature of nickel subsulfide-dependent DNA damage in an effort to qualitatively and quantitatively characterize its effects relative to other particulate-associated toxic metals.

Rationale

The chemical mechanisms involved in toxic metal interactions with biologically important molecules are instrumental in the pathophysiology accompanying the signs and symptoms of toxicity. Although directly induced chemical mechanisms are commonly expected in metal toxicity syndromes, it is clear that indirect mechanisms are also important. For example, toxic metals may cause direct damage through competitive binding interactions which inhibit enzyme activity or indirect damage through free radical formation, resulting in altered signal transduction pathways or cell damage and apoptosis. Still

other mechanisms of toxic metal pathology extend beyond the scope of these direct and indirect effects and occur through limiting availability of essential nutrients, thus inducing deficiencies. Since direct and indirect mechanisms are not adequate terms to characterize a pathological process that occurs as a consequence of induced nutritional deficiencies, we propose this newly defined molecular mechanism be termed “consequent.” The original meanings of the term coincide with the intended new meaning in a number of regards. “Consequent” indicates a natural effect or result; logically correct or consistent, and the second half of a ratio, something which is particularly appropriate in the case of mercury:selenium interactions since mercury toxicity has little to do with mercury exposure but is, instead, almost entirely dependent on the Hg:Se ratio. However, this mechanism is not limited to mercury toxicity, but appears to be a unifying characteristic of many metal toxicities.

The consequent mechanism of metal toxicity can either involve displacement or sequestration of vital trace metals. For example, a major mechanism of zinc intoxication arises from the zinc ion’s absorption across the luminal membrane of gut epithelial cells in preference to copper ions. Zinc and copper compete for the same binding sites on the gut epithelia. In the presence of excessive amounts of dietary zinc, intestinal absorption of copper becomes compromised, the body eventually becomes copper-deprived, and enzymes that depend on copper cease to function. Thus the chief physiological effect of chronic zinc intoxication is depletion of intracellular copper stores, but pathological syndromes associated with zinc toxicity arise because of the consequent loss of copper-dependent enzyme activities.

This research specifically addresses the consequent mechanism of metal toxicity involving mercury and selenium. Selenium performs essential functions in enzymes that require it to perform their numerous physiologically important roles. These enzymes are normally present in all cells of all animal life, but are especially prominent in brain and certain hormone-producing tissues. Because mercury has such high binding affinities for selenium, mercury selenides form that make selenium biologically unavailable. Mercury toxicity occurs at blood levels of 1–2.5 μM , levels approximating the normal selenium concentration in blood. Although the total selenium in the tissue remains at near-normal or even increased concentrations, the selenium sequestered in mercury selenides is biologically unavailable. In the absence of selenium, enzymes that depend on selenium cease to function. Thus, similar to zinc toxicity, the pathological syndrome associated with mercury toxicity appears to arise because of a loss of selenium-dependent enzyme activities. However, the consequent selenium deficit may have previously been overlooked because selenium present as HgSe can be released by acid digestions and show up on analysis, making it appear as if selenium availability in the tissues was unimpaired.

The ability of selenium compounds to decrease the toxic action of mercury has been established in all investigated species of mammals, birds, and fish (1, 2). Selenium and Hg influence each other’s bioavailability (3), toxicology (4), and remediation (5, 6). Several studies suggest an important role of Se in the bioaccumulation of Hg in fish (7–9). Fish tissue Hg is inversely related to the abundance of Se present in the ecosystem (6, 10). Selenium supplementation of lake waters in Sweden resulted in a 75%–85% reduction in Hg levels of fish over a 3-year period (5). Elimination of Se-rich discharges of fly ash to an artificial lake caused a steady increase in Hg concentrations (11, 12). Turner and Rudd (13) studied the uptake of radiolabeled Se and Hg into several fish species, zoobenthos, and plankton in experimental enclosures in a mercury-contaminated lake in northwestern Ontario. Their studies showed that aquatic biota bioaccumulate selenium rapidly with a concomitant reduction in accumulation of mercury. The major sink for mercury and selenium was found to be the sediment. Studies such as these confirm the importance of Se-dependent Hg retirement from aquatic ecosystems. In this regard, important contributions to mercury selenide formation are likely to occur in plants, bacteria, protozoa, crustaceans, arthropods, and mollusks. Additionally, unlike higher organisms, these creatures do not have an acidic gut environment. It is, therefore, assumed the HgSe will pass through its digestive tract and be deposited in the silt. Therefore, at each level of the aquatic food chain, the mercury selenide present in the body mass of the prey would be expected to be far less bioavailable for absorption by the predator. Reduced mercury

bioavailability is expected to result in less bioaccumulation in fish consumed by humans. Interactions between toxic doses of inorganic Hg and toxic doses of selenite in rodents have been thoroughly examined and described as “the mutual alleviation of toxicity through formation of inert Hg–Se complexes.” It has also been shown that marine mammals, having the highest mercury burdens in the marine biota, are able to detoxify methylmercury by formation and storage of HgSe in the liver. As much as 53% of the total mercury in the liver of ringed seals occurs as HgSe (14). Numerous studies demonstrate that the selenium naturally present in foods provides potent protection against mercury toxicity. Since mercury sequesters selenium and reduces its bioavailability for formation of essential selenium-dependent enzymes, additional selenium appears to prevent mercury toxicity by maintaining normal selenoenzyme synthesis. For these reasons, measuring the amount of mercury present in the environment or food sources may give an inaccurate estimation of health risks if the protective effects of selenium are not also considered.

Another area of concern regarding exposure to toxic metals that will be investigated in this study involves the risk associated with exposure to inhaled nickel subsulfide-containing particulates. Nickel and other reactive oxygen species (ROS)-forming metal ions are present in varying distributions in combustion-derived particulates such as residual oil fly ash (15, 16). Therefore, these elements primarily operate through indirect molecular mechanisms of toxicity since the ROS formed by these elements causes the molecular damage associated with their toxic effects. Respiratory and cardiovascular effects of exposure to these airborne particulates are associated with formation of ROS occurring at the particle interface or from water-soluble transition metal ions arising from the particulates. However, there may also be direct mechanisms of molecular interactions that may accompany exposure to certain of these elements.

An earlier study performed at the EERC applied an assay that provides a sensitive and rapid assessment of ROS-generating capacity inherent in the materials analyzed (17). Electrophoresis through agarose gels separates the undamaged supercoiled DNA form from the relaxed form that arises when ROS-dependent damage creates “nicks” in the DNA that interrupt the tightly coiled native structure. The native supercoiled structure has a more compact hydrodynamic diameter that migrates through the matrix of the gels at a greater speed than the damaged form. The Φ X174 DNA that is used has a consistent level (<20%) of relaxed form when it is received. Exposure to materials that produce free radical results in increased amounts of ROS-dependent damage, as seen in Figure 1.

Using this method, potential health hazards associated with exposure to respirable particulate materials may be qualitatively and quantitatively assessed. Distinctions in the ROS-forming capacity of different molecular forms of the chemical species present in these particulates are just as important as the mass quantities of the potentially toxic elements. For instance, distinctions in solubility and stability of various forms of nickel influence cellular uptake rates as well as kinetics of ROS formation (18). As a result, more severe consequences are associated with pulmonary exposure to chemical species such as nickel subsulfide than with nickel oxide (15, 16). This correlates well with analytical observations that the damage induced by nickel subsulfide is not only quantitatively greater, but also qualitatively different than that induced by other nickel species. The current study assesses the DNA damage induced by nickel subsulfide to that caused by various other ROS species in an effort to define and characterize the molecular interactions that are responsible for the greater pathological effects of nickel subsulfide versus other molecular forms of nickel.

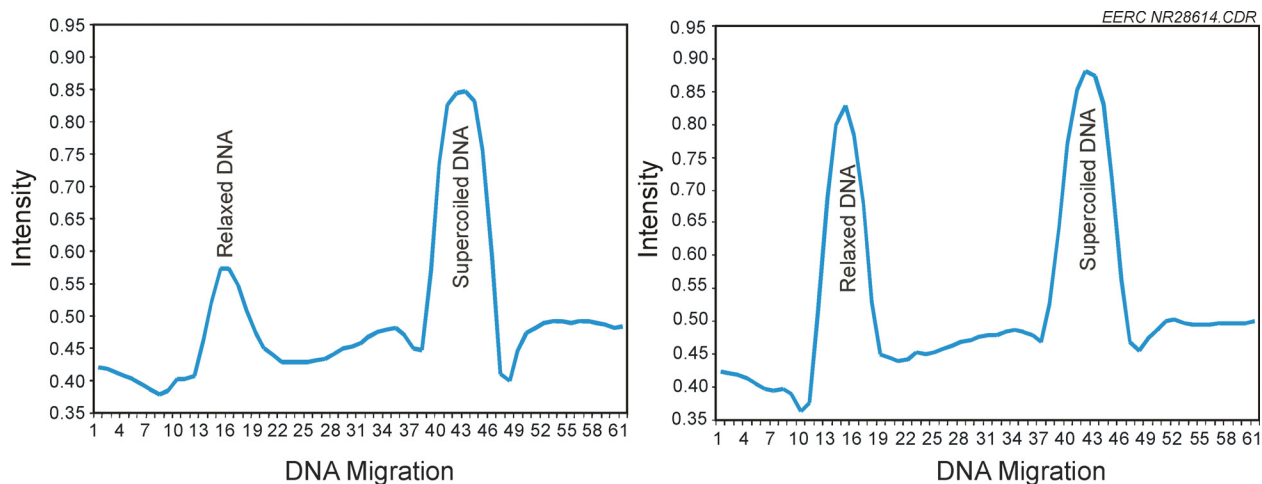


Figure 1. Comparison of electrophoretic migration patterns of normal bacteriophage $\Phi X174$ DNA (left panel) and $\Phi X174$ DNA that has been exposed to fly ash (right panel).

Approach

This project examines the molecular mechanisms responsible for the toxic effects of metals known to be biologically hazardous. Our first task is designed to test the hypothesis that selenium contributes to diminishment of the biological availability of methylmercury in aquatic food webs through formation of insoluble and biologically unavailable mercury selenides, resulting in long-term retirement of mercury from actively cycling pools in the environment. Likewise, our second task will test the hypothesis that the formation of HgSe in the cell contributes to the toxic effects of methylmercury by sequestering necessary selenium required for selenoenzyme synthesis. Our third task will test the hypothesis that nickel subsulfide-dependent DNA damage occurs through a molecular mechanism that is distinct from other free radical-generating metal species.

S.A.M.P.L.E. (Selenium Analysis in the METAALICUS Project Lake Ecosystem)

The objectives of this project are to investigate the potential influence of selenium on mercury dynamics in an extremely well-defined aquatic ecosystem. We are enhancing the METAALICUS (Mercury Experiment to Assess Atmospheric Loading In Canada and the United States) study by determining the selenium concentrations of representative aquatic biota samples in the food web and will correlate our results with total mercury and methylmercury analyses already being obtained as part of that study. METAALICUS is a whole-ecosystem experiment in which mercury loading to a headwater lake and its watershed is being experimentally manipulated. Mercury is being added in distinct forms of stable, nonradioactive isotopes of inorganic mercury. Different mercury stable isotopes are being added to the upland, wetland, and lake surface to determine the relative contributions of these sources to fish mercury levels. The mercury concentrations are being tracked in all major compartments in the lake, watershed, and atmosphere. Our effort in this project is to examine the selenium content in selected fish and invertebrate samples and investigate correlations between endogenous selenium concentrations with the mercury concentrations present in these same samples.

Identification of Mercury Selenide in Tissues

Utilizing tissue samples from experimental animals that have been chronically exposed to large quantities of dietary mercury and tissue samples obtained from whales and squid, we are applying x-ray absorption fine structure (XAFS) and electron microscopy methods to identify and quantify HgSe species in mercury-rich tissues. XAFS has become the technique of choice for determinations of trace element speciation in the complex systems that are typically encountered in environmental, geological, and biological sciences. This direct and nondestructive synchrotron-based method complements analytical determinations of elemental concentrations by providing information as to how an element occurs in the material under investigation. Direct measurement of HgSe in tissues will support the hypothesis we have proposed and will definitively show mercury's role in inhibiting selenoprotein synthesis through sequestration of selenium.

Mechanisms of Nickel Sub sulfide-Mediated DNA Damage

Nickel subsulfide-dependent DNA damage appears to cause DNA damage that is distinct from that caused by other free radical-generating metal species. To evaluate ROS-dependent damage from insoluble forms of metalloparticulates, supercoiled circular DNA from bacteriophage Φ X174 DNA has been used as a test substrate in a highly sensitive monitoring assay. Samples of National Institute of Standards and Technology (NIST) standard particulate materials, nickel subsulfide, and nickel oxide were extracted using 1 M NaOAc–0.5 M HOAc at pH 5 at 25°–28°C, followed by exhaustive rinsing with deionized H₂O. DNA substrate suspensions in aqueous solutions were exposed to equivalent mass quantities of these insoluble residues in multiple parallel samples that were exposed and processed identically. Because of its compact hydrodynamic diameter, undamaged supercoil DNA moves more rapidly through a 0.6% agarose gel during electrophoresis than the relaxed form that occurs following ROS-dependent damage. The relative quantities of DNA migrating as either form is easily visualized using ethidium bromide following electrophoretic separation.

Progress

Samples of zooplankton from the METAALICUS study lakes that were collected in early, mid-, and late summers of 2000–2006 have been received and are being assessed for selenium content. Selenium concentrations in zooplankton samples collected during the summer of 2000 from Lake 658, which was experimentally supplemented with labeled mercury, were measured at 2.67 ± 0.36 , while those collected during the same time periods from the untreated control Lake 240 were similar at 1.99 ± 0.27 . Analysis of samples collected during subsequent years is currently ongoing.

Tissues have been obtained from rats exposed to diets containing MeHg, as well as from beluga and killer whales. Preliminary work with scanning electron microscopy has been performed, and new methodologies employing apparatus that enable samples to be analyzed in situ have been developed. In order to determine the molecular structure of Hg and Se present in tissues from these sources, XAFS spectroscopy has been applied. The bulk of mercury present in these tissues occurs as HgSe in a hextetrahedral conformation, particularly as Hg concentrations increase.

Exposure to nickel subsulfide rapidly and completely eliminates undamaged DNA and results in quantitative conversion into the damaged DNA form as well as a third form that has a migration rate during electrophoresis that is intermediate between the damaged and undamaged forms (Figure 2). In collaboration with the Mayo Foundation Molecular Core Facility, nickel subsulfide-dependent DNA

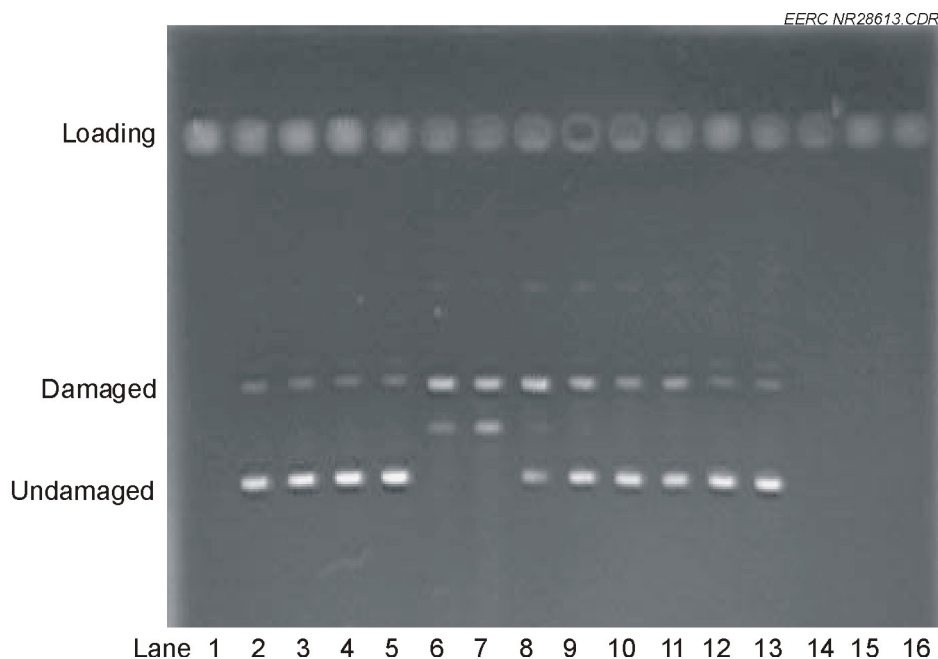


Figure 2. Comparison of migration patterns of untreated DNA (Lanes 2–5, 12, 13) to DNA treated with nickel subsulfide (Lanes 6 and 7) or nickel oxide (Lanes 8–12). Lanes 1 and 14–16 were not used.

damage is being characterized. The rate and degree of damage is many times greater than other forms of and differentiated from the damage caused by other forms of nickel present in inhaled airborne particulates. Distinctions in form and mechanisms of nickel subsulfide-dependent molecular damage to DNA is being assessed in comparison to defined effects of other DNA treatments using differential chromatography studies. Initial studies of the analysis of time- and dose-dependent DNA damage from exposure to nickel subsulfide have been performed that demonstrate the damage arising from nickel subsulfide exposure happens far more quickly and more extensively than the damage that accompanies DNA exposure to reactive oxygen species. As shown in the illuminated ethidium bromide figure depicted in Figure 2, nickel subsulfide-dependent damage gives rise to a novel DNA form that migrates at an intermediate.

Quality Objectives

The quality objective of Task 1 is to produce analytical results of selenium in selected samples from various organisms collected from the aquatic ecosystems that comprise the METAALICUS study. The analyses were performed by the EERC Analytical Research Laboratory (ARL) alongside appropriate quality control samples and certified standard reference materials. The results are expected to provide sufficient data to evaluate the influence of selenium on mercury accumulation.

The quality objective of Task 2 is to produce analytical results of HgSe particulate analysis by XAFS and electron microscopy methods in order to identify and quantitate the formation of these HgSe species in mercury-rich tissues obtained from experimental animals and marine mammals. The identification of the HgSe species will be based on prior reports of HgSe characterization and available standards.

The quality objective of Task 3 is to produce repeatable results of a DNA damage technique which comparatively assesses the inherent ROS formation rates of various compounds. The identification of open coil versus potentially constricted loop forms will be attained by direct comparison of migration behavior of nickel subsulfide-treated DNA with DNA supercoil treated with restriction enzymes to prepare authentic open-coil DNA migration patterns of ligated forms.

Measurement/Data Acquisition

The methods used in this project employ appropriate methods for analysis according to established protocols in place at the EERC. All sampling procedures, instrument calibrations, and quality control (QC) considerations are included in the protocols. Selenium analysis (Task 1) is being performed using hydride-generated atomic fluorescence according to established analytical protocols of the ARL. Mercury selenide species (Task 2) will be identified by using electron microscopy methods according to established analytical protocols of the Natural Materials Analytical Research Laboratory. The DNA damage technique (Task 3) will be performed in the Mayo Foundation Molecular Core Facility, following established and appropriate analytical protocols.

Assessment and Validation

The analysis data for the standard protocols used in this project indicate acceptable analytical accuracy and precision. Certified reference materials and control sample analytical results are within the expected ranges. Using standard statistical analysis, the numbers of samples used for each task provides statistically meaningful results. XAFS spectra of mercury and selenium species are compared to authentic molecular forms used as analytical standards. Multiple spectra are collected from each sample, and mercury and selenium analysis independently confirms HgSe formation and distribution.

Status

To examine selenium-dependent effects on mercury bioaccumulation in the experimental and control lakes of the METAALICUS research project, selenium contents of samples of zooplankton biomass are being examined. Appropriate samples have been identified among the samples obtained from these lakes that are currently being analyzed.

Methodological development has progressed in Task 2, and preliminary analysis of whale tissues has been initiated. XAFS has confirmed HgSe species are present in tissues to be tested, and analysis of mercury selenide forms is ongoing in both in situ and ex vivo isolates from these tissues. Digestion procedures that degrade materials other than HgSe have been studied and are being developed for this project.

Studies of nickel subsulfide-mediated DNA damage are under way, and standardization of sample-handling protocols for the EERC and Mayo Clinic's molecular biology core facility has been developed. Restriction enzymes appropriate for ligation of Φ X174 DNA have been identified and will be used in preparation of open-coil DNA for comparison to nickel subsulfide-treated DNA.

Potential Applications and Benefits

Increased information regarding the effects of selenium bioavailability on methylmercury bioaccumulation will help regulatory agencies interpret differences in methylmercury concentrations in freshwater fish. Since selenium's geologic distribution can vary dramatically over short distances, lake selenium availability has the potential to cause considerable differences in mercury bioaccumulation rates between even neighboring lakes that may have no other perceptible differences in atmospheric deposition or other innate influences. Selenium-dependent mercury retirement will contribute to making previously deposited mercury unavailable, leaving only the most recently deposited mercury in a bioavailable condition. If selenium-dependent mercury retirement is occurring, it will also change current understanding of the global mercury cycle, particularly regarding input-output mass balance results.

The study of the molecular mechanism of nickel subsulfide-dependent DNA damage may provide important information for regulatory agencies to consider. If the mechanism occurs through direct adduct formation rather than through indirect damage from reactive oxygen species, it will guide regulatory requirements for limiting human exposure.

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