Applications of X-ray Absorption Spectroscopy in Life Sciences

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Grand Challenges Canada

Beamlines and their personnel

Overview

• Why use X-ray absorption spectroscopy in life sciences?
• X-ray absorption spectroscopy combined with X-ray fluorescence imaging
• Case studies:
  ◦ Arsenic in plants
  ◦ Mercury in zebrafish as vertebrate model
  ◦ Mercury in human brain
  ◦ Arsenic, selenium and Bangladesh
Synchrotron Facilities in Canada and USA
(those used in my talk!)

CLS:
Canadian Light Source
(Saskatoon, SK)

SSRL:
Stanford Synchrotron Radiation Lightsource
(Stanford, CA)

APS:
Advanced Photon Source
(Argonne, IL)

http://johomaps.com/na/na2.html

Sources of Metals in our Environment

Natural sources

Manmade sources
Sources of Human Exposure to Metals

- food
- drinking water
- contact
- inhalation

Elements in Humans (and other organisms)

**Essential elements**
- copper
- selenium
- molybdenum
- iron
- zinc

**Toxic elements**
- mercury
- cadmium
- arsenic
- lead

Just about all metals are toxic at high levels.
Chemical form matters!

**Example: Arsenic**

- Arsenic is infamous as a poison
  - *e.g.* Lewisite – war gas known as “dew of death”

- However, not all arsenic is poisonous!
  - *e.g.* Arsenobetaine (0.02 wt% in seafood) is not toxic at all

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**X-ray Absorption Spectroscopy in Life Sciences**

- Chemical form affects the element’s properties, *e.g.*
  - Solubility and mobility in groundwater
  - Bioavailability to organisms
  - Toxicity or benefit to higher organisms including humans

- Samples are often heterogeneous, *e.g.*
  - Organisms, tissues, cells
  - Food, soil, sediment

- Need to know the chemical form of a potentially toxic or beneficial element in a complex matrix
  - X-ray absorption spectroscopy can do this
X-ray Absorption Spectroscopy in Life Sciences

- XAS gives local structural information around central absorbing atom
  - Atomic property so no confusion over which element

- Due to X-ray properties, XAS can be used for almost any matrix:
  - Purified protein solution (simple)
  - Biological tissues, sediments, etc. (complex)

- Analyzes all forms of the element with no “hidden” phases, e.g.
  - Crystalline or amorphous solids, aqueous solutions, gases…
  - Can provide information on mixtures of chemical species

- XAS is very amenable to investigating metals and other elements in biological and environmental samples
  - One of few “in situ” probes, little pre-treatment required

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X-ray Absorption Spectroscopy: Direct and Potentially Non-Destructive

Conventional analysis → Molecular information may be lost

X-ray absorption spectroscopy → Direct probe, molecular form intact
Imaging Chemical Species in Biological Samples

- Many complex samples have spatial structure
- Questions we may want to answer:
  - How is an element distributed?
  - What is an element’s chemical form in a particular location?
  - How is a chemical species distributed?
- We would like to do this:
  - For dilute levels of elements (use fluorescence)
  - Sometimes on intact living specimens
- Answer these questions using
  - X-ray fluorescence imaging in combination with
  - X-ray absorption spectroscopy

X-ray Fluorescence

- An X-ray fluorescent photon is emitted when an electron is absorbed
- Measuring fluorescence on a thin/dilute sample
  - Is equivalent to measuring absorbance
  - Is much more sensitive to dilute species
- It is also the basis of X-ray fluorescence imaging
X-ray Fluorescence Imaging


Elements Accessible by X-ray Fluorescence Imaging

X-ray Fluorescence Imaging

X-ray fluorescence emission spectrum from energy-dispersive detector

Human cerebral cortex, individual poisoned through skin exposure to dimethylmercury.
Canberra LeGe detector, analog electronics, Gaussian shaping amplifier, shaping time 2 μs, incident X-ray energy 13.200 eV. SSRL 9-3.

Pathie, Pickering, Korbas, Hackett & George
Chemical Reviews 114(17):8499-8541 (2014)
X-ray Fluorescence Imaging

- Fixed energy above absorption edge(s) of elements of interest
- Micro-focused (“pencil”) beam
- Spatially raster sample in beam
- Measure fluorescence emission spectrum at each pixel
- Produces elemental maps
- Can be combined with X-ray absorption spectroscopy to provide chemical information

Spatial Resolution

- Spatial resolution of X-ray fluorescence imaging should be tuned to the object of interest
- Microfocus optics (to make a small beam)
  - (Apertures)
  - Kirkpatrick-Baez (K-B) mirror pair
  - Glass capillaries
  - Zone plates

Pushie, Pickering, Korbas, Hackett & George
Chemical Reviews 114(17):8499-8541 (2014)
Near-edge (XANES) is sensitive to chemical form

Simultaneous Spatial and Chemical Information

Two routes to information

1. X-ray Fluorescence Imaging Plus Micro-XAS

2. Chemically Specific Mapping (Tune incident energy to peaks)
Micro-XAS

- Complete fluorescence map
- Select pixel of interest
- Scan incident energy
- Also called μ-XAS, μ-XANES, μ-XAFS

- Gives complete spectrum, but at limited points

Pushie, Pickering, Korbas, Hackett & George
Chemical Reviews 114(17):8499-8541 (2014)

Chemically Specific Imaging
Map at two or more energies to generate chemical species maps

X-ray fluorescence at different incident energies:

E Energies
E1 E2

Concentrations
Arsenite Arsenate

Pickering et al., PNAS 97(20) 10717-10722

Transmittance Effective thickness
Chemically Specific Imaging vs. XFI

Differences lie in the incident energy

Chemical Specific Imaging
Energy at the edge:
Image chemical species

X-ray Fluorescence Imaging
Energy above the edge:
Image element(s)

Choice of Chemically Specific Imaging

- X-ray Fluorescence Imaging plus micro-XAS:
  - Gives entire spectrum at selected points
  - May miss spatial detail
  - Longer dwell time at those pixels

- Chemically Specific Imaging:
  - Need to know which species to look for
  - Need good spectral contrast
  - Gives complete spatial maps of each species
  - Shorter dwell times
Case Study: An Arsenic-Loving Fern


- **Pteris vittata:** a hyperaccumulator of arsenic
  - Takes up, stores and tolerates arsenic in tissues
  - High tissue concentration compared with soil
  - Can store up to 2% dry weight As

- Arsenic is a major environmental problem in many countries

- **Pteris vittata** shows potential in arsenic *phytoremediation*
  - Use of plants to remove arsenic from contaminated areas
  - (Either *Pteris vittata* itself or its pathways in engineered plants)

Phytoremediation

Soil contaminated with a metal – how do we clean this up?

Grow plants which take up the metal…

Metal-loving plant
Phytoremediation

Harvest the plants and remove the contamination!

$Pteris\ vittata$ - Questions

- Unanswered Questions –
  - What chemical forms of arsenic are present?
  - What biotransformations of arsenic are taking place?
  - Where does biotransformation occur?
  - How does the plant avoid poisoning itself?

- Need a direct probe of arsenic chemical form within living plant tissues
  - X-ray absorption spectroscopy to determine speciation
  - Chemically specific mapping to determine localization
Pteris vittata – X-ray absorption spectroscopy

Use As K-edge XAS of standards as a fingerprint of chemical form

![Graph showing normalized absorbance vs energy for different As species (As(3S), As(OH)3, [H2AsO4]).](image)

Different locations in fern show different As chemical species

Leaf (arsenite)

Stem (mixture)

Root (arsenate)

Use chemically-specific imaging to provide more detail
**Pteris vittata**: Chemically Specific Mapping of Leaflet (Pinna) Tip

Arsenate localized in leaf vein, arsenite high in leaf blade

Transport vessels reveal arsenate and arsenite

Arsenate visible in xylem
Reproductive Tissues Show Exclusion of Arsenic

Sporangia: arsenic in paraphyses, not spores

Gameophyte: arsenic absent from reproductive area

What About Sulfur?

- Arsenic loves sulfur coordination
- Cells are full of available sulfur
- Why isn't arsenic coordinated by sulfur???
  Well actually, maybe it is…

- Bulk near-edge and EXAFS shows borderline evidence for thiolate-coordinated species
- Use 3-component Chemically Specific Imaging to attempt to localize As-thiolate species
Use 3-Energy Chemically Specific Imaging

Collect images at energies sensitive to arsenic chemical species

As(GS)₃
As(OH)₃
[H₂AsO₄]⁻

Arsenic-Sulfur Species Revealed Near Vein

Vein
Mid-vein
Thickness
Blade
Optical

Sulfur may be implicated in reduction

Arsenite: stored in blade
Arsenate: in veins (xylem)
Arsenic-thiolate: surrounds veins

As-thiolate
Arsenate
Arsenite
Arsenic in Fern - Summary

- **Sporophytes:**
  - Arsenate is transported in the xylem to the leaves
  - In leaves, arsenite is stored at high levels
  - Thiolate-coordination may be implicated in reduction

- **Reproductive tissues:**
  - Arsenic is excluded


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Mercury

In collaboration with Graham George, University of Saskatchewan
Mercury

- Mercury is one of the most toxic elements to which humans are commonly exposed
- Acute exposure of organomercury leads to severe consequences, especially in children or fetuses
  - microcephaly
  - cerebropalsy
  - seizures
  - mental retardation
  - blindness
  - quadriplegia

Minamata Japan

- Caused by dumping of mercury-containing industrial waste by Chisso Corporation into Minamata Bay in the 1950’s and 60’s
- Local fish became heavily contaminated with methylmercury compounds
- First victim was a 5-year old girl in 1956
- Final death toll approached 2,000 people
- Discovery that organo-mercury compounds affect foetal development

* http://www.chisso.co.jp/english/index.asp
(http://www.jnc-corp.co.jp/english/)
Mercury in our food

Fish are a significant source of mercury in our diets and a major source of potentially neurotoxic methylmercury species.

How much mercury in your diet is safe?

In the USA, the FDA, the AHA and the EPA disagree
- EPA: “eat no more than two fish meals a week” “do not eat fish that are high in mercury”
- FDA: “nutritional benefits of eating fish outweigh the risks from mercury”
- AHA: “healthy people should eat fish at least twice a week”

How much fish in our diet is safe?

Recommended limits are mainly based on epidemiological studies

Pregnant women with high dietary Hg from seafood
Measure Hg in hair and blood
Follow pregnancy then development of child
Do developmental deficiencies correlate with Hg levels?

Hg Source: Mainly pilot whale meat and some fish
Correlation: Yes!

Hg Source: Shark as “fish and chips”
Correlation: No

Hg Source: Fish
Correlation: No

New Zealand
Seychelles
Faroes
Banning Fish will Impact World Health

The United Nations Food and Agriculture Organization estimates that over one billion people depend on marine fish as primary daily nutrition.

If the West passes legislation declaring fish unsafe to eat then other countries may follow suit.

This could significantly and negatively impact world health.

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**Research Goals**

- Despite mercury's importance, mechanisms by which it exerts its toxic effects remain unknown.
- Understand effects of mercury at the molecular level:
  - How is it transported?
  - Where is it localized and is it mobile?
  - How does molecular form affect these properties?
- Use X-ray absorption spectroscopy and X-ray fluorescence imaging applied to:
  - Developing vertebrates (zebrafish)
  - Human tissues
Mercury in Zebrafish

- Mercury is well known as a toxic element but different forms show widely different toxic effects
  - Methyl mercury species considered neurotoxic
- Use zebrafish to study how different mercury forms accumulate
  - Zebrafish are a well-established vertebrate model
  - Easy to maintain, quick growth, well characterized staging series


Zebrafish and Methylmercury L-Cysteine

- Treat with 100 μM CH₃Hg(L-Cys)
  - 24 h exposure
  - 3.5 dpf

- Organic mercury accumulates preferentially in outer layers of eye lens

Mercury in Zebrafish: Exposure

<table>
<thead>
<tr>
<th>Inorganic Hg</th>
<th>Organic Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mercuric chloride</td>
<td>1 μM Methyl mercury chloride</td>
</tr>
<tr>
<td>Mercury bis-L-cysteinate</td>
<td>100 μM Methyl mercury L-cysteinate</td>
</tr>
</tbody>
</table>

## Chemical Form Results

### Eyes & Brain

<table>
<thead>
<tr>
<th>Inorganic</th>
<th>Organic</th>
</tr>
</thead>
<tbody>
<tr>
<td>HgCl</td>
<td>Hg(L-Cys)$_2$</td>
</tr>
<tr>
<td>0 µg/cm$^2$</td>
<td>0.06 µg/cm$^2$</td>
</tr>
</tbody>
</table>

### Liver & Kidney

<table>
<thead>
<tr>
<th>Inorganic</th>
<th>Organic</th>
</tr>
</thead>
<tbody>
<tr>
<td>HgCl</td>
<td>Hg(L-Cys)$_2$</td>
</tr>
<tr>
<td>0 µg/cm$^2$</td>
<td>0.1 µg/cm$^2$</td>
</tr>
</tbody>
</table>

Conclusions from Zebrafish

- Chemical form plays an important role in toxicity
- Preferential accumulation:
  - Organomercury in eye lens epithelium, skeletal muscle, gut tube
  - Inorganic mercury in brain ventricular region
  - Both accumulate in sensory organs and brain
- Organomercury mostly accumulates to higher levels
- Zebrafish: model system to study toxic metals

Mercury, Selenium and Human Brain


ACS Chemical Neuroscience, 2010, 1 (12), 810-818

a University of Saskatchewan
b University of Rochester

Gosia Korbas
### Objective

To investigate the molecular nature of **mercury** and **selenium** in human brain samples

<table>
<thead>
<tr>
<th>Case</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<tbody>
<tr>
<td>Gender</td>
<td>F</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>F</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>29</td>
<td>48</td>
<td>60</td>
<td>76</td>
<td>67</td>
</tr>
<tr>
<td>Cortex</td>
<td>frontal</td>
<td>occipital</td>
<td>occipital</td>
<td>occipital</td>
<td>occipital</td>
</tr>
<tr>
<td>Mercury Exposure</td>
<td>acute poisoning at age 8 yrs</td>
<td>acute poisoning, 10 months to death</td>
<td>fish consumption</td>
<td>fish consumption</td>
<td>none known</td>
</tr>
<tr>
<td>Toxicant</td>
<td>CH$_3$Hg-X</td>
<td>(CH$_3$)$_2$Hg</td>
<td>CH$_3$HgS(thiol)</td>
<td>CH$_3$HgS(thiol)</td>
<td>n/a</td>
</tr>
<tr>
<td>Hg(ppb)</td>
<td>1179</td>
<td>2670</td>
<td>324</td>
<td>120</td>
<td>0.06</td>
</tr>
<tr>
<td>Pathology</td>
<td>severe atrophy</td>
<td>severe atrophy</td>
<td>normal</td>
<td>normal</td>
<td>normal</td>
</tr>
</tbody>
</table>


### Near-edge spectra as chemical fingerprints

![Near-edge spectra](attachment:image.png)

**Nano-HgSe Shows Damped Features**

- Spectra are similar compared with crystalline
  - Similar maxima, but much less pronounced
  - Indicates greater disorder due to truncation effects

![Graphs showing normalized absorbance of Hg and Se for crystalline and nano-particulate forms.](image)


**Mercury in brain**

Three main Hg species

- Total Hg: 30 μM
  - Hg(SR)₂ 29%
  - HgSe 71%
  - nano-HgSe

- Total Hg: 22 μM
  - CH₃HgCys 55%
  - Hg(SR)₂ 29%
  - nano-HgSe

- Total Hg: 1.4 μM
  - CH₃HgCys 65%
  - Hg(SR)₂ 35%
  - nano-HgSe

Slide courtesy of Gosia Korbas

NX 2015
Selenium in brain

Three main Se species

- nano-HgSe
- RSeSR'
- RSeR'

Total Se: 26 µM
   Org-Se: 3.9 µM

Total Se: 11 µM
   Org-Se: 3.6 µM

Total Se: 3.8 µM
   Org-Se: 3.3 µM

Total Se: 3.4 µM
   Org-Se: 3.4 µM

Total Se: 3.6 µM
   Org-Se: 3.6 µM

Slide courtesy of Gosia Korbas

EXAFS Shows Evidence for HgSe Species

Extended X-ray absorption fine structure (EXAFS)
Fourier transforms

3.5 Hg-Se @ 2.63 Å
3.5 Se-Hg @ 2.61 Å

Hg and Se are Co-localized

CASE 2

Majority of Hg and Se co-located in grey matter

X-ray fluorescence imaging

Selenium and Mercury in Brain: Summary

- Nano-HgSe seen in all higher exposures
  - Likely an inert species involved in detoxification
- Total brain Hg is not good measure of Hg toxic potential
- Se distributed between
  - organic selenium: remarkably constant
  - nano-HgSe: increases with Hg levels
- "Organic" brain average Se level is not depleted
- Se may redistribute from other structures to bind Hg

Case Study:
Arsenic and Selenium in Bangladesh

Hg Sn Tl Pb
Lanthanides Actinides

Mn Co Fe Zn Ni Cu
Ga Ge Kr Br
Sb Xe Te I
Al PSi Ar Sc I
BN CN e OF
He

Bi Rn Po At
Ti Sc Cr Mn V Co Fe Ni Cu
Zr Yb Tc Nb Rh Ru Pd Ag Cd
In Sn Sb Te I Xe
Cs Ba La Hf Ta W Re Os Ir Pt Au Hg Ti Pb Bi Po At
Fr Ra Ac

Selenium is an essential trace element

Arsenic has no confirmed biological function


Stanford Synchrotron Radiation Lightsource
**Two Wrongs that do Make a Right: Arsenic and Selenium**

Moxon (1938) – Arsenite completely protected rats fed upon selenized wheat

Fed 11ppm Se

60 days

60 days

100 days

5ppm arsenite in drinking water

All alive

All dead

“The feeding of arsenic to livestock to prevent selenium poisoning is not recommended on the basis of these results...”

A lethal dose of arsenite (or selenite) can be completely counteracted by an equal and otherwise lethal dose of selenite (or arsenite).

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**Arsenic and Selenium in Bile**

- Inject rabbit with arsenite and selenite
- As and Se: excreted in bile only when both are present
- As:Se 1:1 in bile
- Suggests an arsenic-selenium molecule responsible for mutual detoxification

Arsenic-Selenium Antagonism

- Bile As and Se K near-edge spectra identical with model synthesized from arsenite, selenite and glutathione

![Graph showing normalized absorbance of As and Se in bile and model](image)


Arsenic-Selenium Antagonism

- Bile As and Se EXAFS used to structurally characterize species in bile

<table>
<thead>
<tr>
<th>Species</th>
<th>EXAFS</th>
<th>Distance (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>As EXAFS:</td>
<td>2 As-S</td>
<td>2.25 ± 0.02 Å</td>
</tr>
<tr>
<td></td>
<td>1 As-Se</td>
<td>2.32 ± 0.01 Å</td>
</tr>
<tr>
<td>Se EXAFS:</td>
<td>1 Se-As</td>
<td>2.31 ± 0.02 Å</td>
</tr>
</tbody>
</table>

As-Se Species Identified in Bile

- An unusual molecule with 1:1 Se:As - the seleno-bis(S-glutathionyl) arsinium ion

Energy minimized DFT optimized geometry


Arsenic water contamination

Amini et al., 2008, Env. Sci. Technol. 42:3669-75
Arsenic Poisoning in Bangladesh

- In Bangladesh & parts of India, tube wells are contaminated with arsenic
- Leads to “arsenicosis”
  - Dermatitis and skin disorders
  - Malignant tumors
  - Death
- 25% of all deaths in affected areas now due to arsenicosis
- Between 35 and 85 million people are affected*

* Estimated by World Health Organization

Percentage of wells measuring >50 ppb As in water
data from British Geological Survey Report WC/00/19

Arsenic Poisoning in Bangladesh

- Puzzles:
  - Not all people drinking from same well get sick
  - Other areas in the world have high arsenic but not the same symptoms

- Clues:
  - Bangladesh is low in dietary selenium
  - Very low selenium levels in livers of victims
  - Other arsenicosis areas also have low selenium
A Selenium-Arsenic Hypothesis

- Formation of \([(\text{GS})_2\text{AsSe}]^–\) is a mechanism to that serves to mutually detoxify As and Se
  - \([(\text{GS})_2\text{AsSe}]^–\) is rapidly excreted to bile
- Selenium is an essential trace element
  - For every atom of As removed, one atom of Se is lost too
- Diet in arsenicosis areas is low in Se
  - Is the arsenicosis an As-induced Se deficiency?
  - Chronic Se deficiency can cause symptoms similar to arsenicosis
- First suggestion of a palliative role for Se in arsenicosis

Clinical Trial of Selenium Supplementation

- Benefits observed in only ~15% of patients
- No statistical demonstration of benefits
- Selenium levels appeared synchronized in a significant number of patients

<table>
<thead>
<tr>
<th>Patients</th>
<th>819</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration</td>
<td>48 weeks</td>
</tr>
<tr>
<td>Location</td>
<td>Upazilla, Bangladesh</td>
</tr>
<tr>
<td>Dose</td>
<td>200 μg Se/day or placebo</td>
</tr>
<tr>
<td>Endpoints</td>
<td>Dermatological</td>
</tr>
</tbody>
</table>

Selenium Treatment of Arsenic Toxicity & Cancers in Bangladesh [SETAC]
Phase III, Double-blind, Randomized, Placebo-Controlled Trial on the Use of Long-term Dietary Selenium in Countering Arsenic Toxicity, Sponsored: NIH/NIH, American Cancer Society
Preliminary Results, Pilot of New Clinical Trial

- Selenium supplement promotes fecal arsenic excretion
- New clinical trial now funded by Grand Challenges Canada

https://youtu.be/D_845FQL0c8

Closing Remarks

- X-ray absorption spectroscopy provides chemical information on heavier elements in the life sciences
  - Often complex, heterogeneous systems
- Combined with X-ray fluorescence imaging, provides spatial resolution of chemical information