Marine Inorganic Biochemistry: From Photoreactive Siderophores to Iodide in Kelp

Frithjof C. Küpper
Overview

• Introduction: Metals in the ocean

Case studies:
• Marine siderophores
• Iodide accumulation in kelp
• Algal genomes
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Transition metals in terrestrial organisms

- First row series: V, Mn, Fe, Co, Ni, Cu, Zn
- Second row series: Mo
- Fe usually very abundant in soil and freshwater ecosystems!
- Concentrations of other metals may vary widely.
# Metals in the Ocean

<table>
<thead>
<tr>
<th>Metal</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mo</td>
<td>100 nM</td>
</tr>
<tr>
<td>V</td>
<td>20 - 35 nM</td>
</tr>
<tr>
<td>Ni</td>
<td>2 - 12 nM</td>
</tr>
<tr>
<td>Zn</td>
<td>0.1 – 8 nM</td>
</tr>
<tr>
<td>Cu</td>
<td>0.6 - 5.7 nM</td>
</tr>
<tr>
<td>Cr</td>
<td>3 - 4.5 nM</td>
</tr>
<tr>
<td>Fe</td>
<td>0.05 – 0.7 nM</td>
</tr>
<tr>
<td>Mn</td>
<td>0.03 – 0.8 nM</td>
</tr>
</tbody>
</table>

Relative abundance of "exotic" biometals

Surface concentrations are often lower than in deeper waters! (Exceptions: Mn, Co)
Metals in the Ocean

Butler A: Acquisition and Utilization of Transition Metal Ions by Marine Organisms.
Science 281, 207-210

Fig. 2. Vertical profiles of the first-row transition metal ions and selected other elements in the North Pacific Ocean. Speciation is not included. Data compiled by Y. Nozaki (2). References for plotted data include: Sc (40), Ti (47), V (3), Cr (42), Mn (43), Fe (44), Co (44), Ni (45), Cu (45), and Zn (45). For a recent review, which includes speciation, see (7).
Fundamental differences between the marine and terrestrial biosphere

- Iron tends to be scarce in the ocean!
- In fact, marine primary productivity is limited in HNLC (high nitrogen, low chlorophyll) regions by lack of iron.
- In the sea, Mo, V, Ni, Zn, Cu are much more abundant than iron!
Strategies of marine organisms to cope with low iron availability

- Highly efficient uptake and recycling systems
- Use of chemical alternatives to iron
Marine metalloenzymes: Mo

- Dimethylsulfoxide (DMSO) reductase
  \[(\text{CH}_3)_2\text{SO} + 2\text{H}^+ + 2\text{e}^- \rightarrow (\text{CH}_3)_2\text{S} + \text{H}_2\text{O}\]

- O atom transfer (sulfoxide / sulfide)

- Membrane-bound or periplasmatic enzyme of marine bacteria
Marine metalloenzymes: Zn, Co and Cd carbonic anhydrases from diatoms

\[ \text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{HCO}_3^- + \text{H}^+ \]

- No homologies to other carbonic anhydrases
- Can substitute Co or Cd for Zn!
- Efficient Lewis acid-type catalysts
- The first documented case of Cd in biology!

F.M.M. Morel \textit{et al.}
The World Ocean, a halogen-rich environment!

- Marine organisms produce a plethora of halogenated natural products
- In many cases, metalloenzymes are involved in the biosynthesis
Marine metalloenzymes: Vanadium (V) haloperoxidases from marine red and brown algae

\[ X^- + H_2O_2 + R-H + H^+ \rightarrow R-X + 2 H_2O \]

- Other peroxidases: heme (Fe) peroxidases in most terrestrial organisms, W peroxidases / oxidoreductases in hyperthermophilic archaea
Vanadium haloperoxididases

- Vanadium (V): vanadate
- Homology to acidic phosphatases

Vanadium haloperoxidases

- A role in the synthesis of halogenated marine natural products (esp. red algae: *Laurencia* sp., *Plocamium cartilagineum*)

Selected examples of halogenated marine natural products.

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Siderophore-mediated metal uptake

Siderophore, *Greek*: “iron carrier”
Marine siderophores

Marine siderophores

• Some well-known structures (e.g. aerobactin), but mostly many novelties

• Mediate prokaryotic Fe / metal uptake in marine systems: availability to eukaryotes not well established yet (e.g. in algal-bacterial symbioses)

• Most eukaryotic algae seem to have plasma membrane-bound ferrireductases

• Several marine siderophores are photoreactive!
Photolysis of aquachelin

**Fe(III)-Aquachelin complex**

![Chemical structure of Fe(III)-Aquachelin complex]

**Photolysis products**

![Chemical structure of photolysis products]

\[ \text{hv} \]

\[ + \text{R} \]

\[ + \text{Fe}^{2+} \]

**Aquachelin A**

**Aquachelin B**

**Aquachelin C**

**Aquachelin D**

\[ K_{\text{cond}}^\text{Fe(III)}' = 10^{12.2} \text{ M}^{-1} \]

\[ K_{\text{cond}}^\text{Fe(III)}' = 10^{11.5} \text{ M}^{-1} \]

Barbeau, Rue, Bruland & Butler, 2001: Nature 413, 409-13
Other cases of photoreactive siderophores

**Petrobactin**

**Aerobactin**

Common feature: \(\alpha\)-hydroxy carboxylic acid moiety

Barbeau *et al.*, 2002: JACS 413, 409–13
Key questions

• Mechanistic aspects of photolysis
• Structure of photoproduct
• How do the physicochemical properties of the photoproduct compare to those of the original siderophore?
• Is photoproduct-bound Fe(III) as bioavailable as Fe(III) bound to the original siderophore?

=> Case study: AEROBACTIN
ESI-MS (positive ion mode): Fe-aerobactin photolysis

Fe-aerobactin photolysis

\[ \Delta m/z = 46 \text{ amu} \]

apo-aerobactin

apo-photoprodut

Fe\(^{3+}\)-photoprodut

Fe\(^{3+}\)-aerobactin

decarboxylation
Quantum mechanical structure optimization of the Ga\(^{3+}\) complexes of aerobactin and its photoproduct

(Spartan 2000 - PM3 level of theory)

(C. J. Carrano)
Summary & conclusions: aerobactin photopродuct

- Photochemistry results in a new ligand of similar physiological properties in bacterial cells
- Photochemistry of Fe-aerobactin yields reactive Fe$^{2+}$, potentially available for a wide range of other organisms and ligands over a transient time span
- Fe bound to the photopродuct can be considered to be of limited access to marine biota
- Oceanographic / ecological implications (this is likely to be relevant only in euphotic open-Ocean conditions, yet not for Enterobacteria also producing aerobactin!)? Is there any evolutionary advantage of producing photoreactive siderophores?

Summary: Siderophore photochemistry

Bacterium

1. **Excretion**
   - Apo-siderophore
   - $+ \text{Fe}^{3+}$

2. **Uptake**
   - Siderophore – $\text{Fe}^{3+}$
   - $h\nu$
   - $\text{Fe}^{2+}$
   - $+ \text{Fe}^{3+}$
   - Siderophore photoproduction
   - $+ \text{Fe}^{3+}$
   - $\text{Fe}^{3+}$-photoproduction

3. **Oxygen**
   - $\text{Fe}^{3+}$
   - $\text{Fe}^{2+}$
   - Siderophore photoproduction

4. **Uptake!**
Algal-bacterial symbioses

- Bacterial symbionts of the dinoflagellate *Gymnodinium catenatum* (D.H. Green)
Isolation of siderophores from culture media of bacterial symbionts

- Vibrioferin
Vibrioferrin

• Previously isolated from *Vibrio parahemolyticus* (enteropathogenic, estuarine bacterium)

• Isolation of an unusual derivative – an unexpected isotope pattern suggested the presence of an element other than the usual C, H, N, O or S

=> *Boronylated vibrioferrin!*
Boronylated vibrio ferrin

(Spartan 2000 - PM3 level of theory)
Boron

• Discovered by Joseph-Louis Gay-Lussac and Louis-Jaques Thénard, and independently by Sir Humphry Davy, in 1808 (isolation of boron by combing boric acid (H3BO3) with potassium)

• Typical oxidation state: +3, stable isotopes: $^{10}_5\text{B}$, $^{11}_5\text{B}$

• In seawater: fairly abundant - about 0.4 mM (but variable) (22nd most abundant element in Earth’s crust, after N and Cu)

• Well-studied in the context of seawater desalination
Boron

• Applications: enamels, borosilicate glass, pyrotechnics, nuclear reactors, boron fibers in engineering

• Very few (< 5) boron-containing natural products known so far, including a quorum sensing molecule

• Boron is an essential trace elements for algae and terrestrial plants!

• Unknown function in eukaryotes
The Boron Story


Amin S, Küpper FC, Green DH, Harris WR, Carrano CJ, 2007: Boron binding by a siderophore isolated from marine bacteria "associated" with the toxic dinoflagellate G. catenatum. Journal of the American Chemical Society 129, 478-479
The Boron Story

momentum distribution. The proposed scenario offers the prospect of engineering optical lattices for the modeling of complex interacting phenomena from the likes of high-temperature superconductivity to magnetic frustration. — ISO


BIOCHEMISTRY

Acquiring a Trace Element

Iron, as the central element in heme cofactors or as part of metal clusters, endows enzymes with the capacity to carry out a much wider range of redox reactions (such as those in respiration and photosynthesis) than is supported by the functional groups of the genetically encoded amino acids. Hence, the acquisition of iron is a highly competitive endeavor, and as ocean supplementation experiments have shown, iron can be a limiting nutrient for the growth of plankton. Nevertheless, marine organisms face a special challenge because iron in an aqueous and aerobic environment of neutral pH is present mostly in insoluble forms. The bacterial solution has been the manufacture and secretion of siderophores, small molecules that chelate Fe(III). Following on their previous identification of a borate-siderophore interaction, Harris et al. provide a fuller characterization of the equilibria in the reaction of B(OH)₄⁻ and vibrioferin, a siderophore of Marinobacter spp. The tetrahedral coordination of B(III) by the pair of α-hydroxy-carboxylate moieties in vibrioferin is highly pH-dependent, and accounting for the protons contributed by the hydroxyls as well as one donated by solvent allowed the authors to assemble the formal binding constants for the multiple borate-vibrioferin complexes. Extending this analysis to the other two types of siderophores—the catecholates and the hydroxamates—revealed that the former are also competent to bind boron whereas the latter are not. Whether any of these capabilities are in fact used by the siderophore producers is as yet unclear, though low-pH environments may be one place to look. — GJC

Fe-vibrioferri is highly photoreactive

- Unlike for aerobactin and all other photoreactive siderophores known so far, photochemistry destroys the Fe-binding backbone of VF

- VF photochemistry generates free reactive iron intermediates which enhance iron uptake of the algal symbiont
Boron, iron and vibrioferroferrin (VF)

VF + Fe$^{3+}$

Excretion

VF–Fe$^{3+}$

Uptake

VF–B

VF

+ Fe$^{3+}$

+ BO$_3^{-3}$

Bacterium

Alga

Uptake!!

Uptake?

Biol. effects?

Fe$^{2+}$

+ VF photoproduct

(no Fe$^{3+}$ binding)

O$_2$

Fe$^{3+}$

“phycosphere”

h$\nu$
Vibrioferrin photochemistry
– a key factor in an algal-bacterial symbiosis


Amin SA, Green DH, Küpper FC, Carrano CJ, 2009: Vibrioferrin, an unusual marine siderophore: Iron binding, photochemistry, and biological implications.- Inorganic Chemistry 48, 11451-8
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Iodine in seaweeds:
A bit of historic background

- Goiter-preventing effects of seaweeds: Known to the Chinese emperor Shen-Nung (third millennium B.C. !)

- Use of burnt seaweeds and sponges as diet supplements for the same purpose: Common in ancient Greece at the time of physician Hippocrates [460-370 B.C.]
Biological model: *Laminaria digitata* (Phaeophyceae)
Iodine as a novel chemical element was discovered in the ashes of *Laminaria*

Courtois, B. (*lu par / read by N. Clément*), 1813:
Découverte d’une substance nouvelle dans le Vareck.
Annales de Chimie 88, 304-310

\[ 2 \text{I}^- + \text{H}_2\text{SO}_4 \rightarrow \text{I}_2 + \text{SO}_3^{2-} + \text{H}_2\text{O} \]

• Strong corrosion of copper flasks
• Emission of violet vapor

⇒ Fr. *IODE* / En. *IODINE*
Greek *ιώδης*, i.e. *violet*
Kelp as the world’s first raw material for iodine production

Re-enactment of traditional kelp harvest, Breton folk festival in Roscoff, Brittany, France

(Gouel Rosko, 1997)
Kelp as the world’s first raw material for iodine production

Re-enactment of traditional kelp burning, Breton folk festival in Roscoff, Brittany, France

(Gouel Rosko, 1997)
Kelp as the world’s first raw material for iodine production

Historic kelp incinerator in Porspoder, Finistère, Brittany, France (June 2004)

Iodine production from seaweeds became a major economic activity in coastal regions of Europe - in particular, in parts of Brittany, Normandy, Ireland and Scotland
Current-day harvesting of kelp – no longer a raw material for iodine production

Kelp harvesters (*goëmoniers*) near Porspoder, Finistère, Brittany, France – use of kelp for alginate extraction (June 2004)
The Breton coast
– where the seaweeds during the pioneering era of iodine research came from

Near Porspoder, Finistère, Brittany, France (June 2004)
The Breton coast
– where the seaweeds during the pioneering era of iodine research came from

Near Porspoder, Finistère, Brittany, France (June 2004)
Iodine accumulation in *Laminaria*

- Laminariales (kelps) are a major biogeochemical pump of iodine!
- *Laminaria* is the strongest iodine accumulator in life

**Still unclear:**

- Chemical form of accumulated iodine?
- Biological significance?
Iodine accumulation in *Laminaria*

- Requirement of an intact cell wall (apoplast)
- Role of hydrogen peroxide and haloperoxidases in iodine uptake
- Iodine efflux upon oxidative stress

Iodine accumulation in *Laminaria*

- Strong seasonality!

Iodine accumulation in *Laminaria*

- Accumulation in cortical tissues

Particle-induced X-ray emission
Elemental map of the meristoderm and outer cortes in a *L. digitata* stipe section

Iodine accumulation in *Laminaria*

Techniques used in this study:

- GC/MS
- Gas-phase particle counters
- Cathodic stripping square wave voltammetry (CSSWV)
- X-ray absorption spectroscopy using synchrotron radiation (XAS)

etc.
Biological XAS at the EMBL Outstation, Hamburg
Biological XAS at the EMBL Outstation, Hamburg
Iodine XAS of *Laminaria* tissues

- Iodide ($I^-$) is the accumulated form (Iodine K-edge)

- Iodide ($I^-$) is the accumulated form of iodine in *Laminaria*!
Iodine metabolism and oxidative stress

- Iodine **uptake** requires low \( \text{H}_2\text{O}_2 \) levels (< 25 \( \mu \text{M} \))
- Higher concentrations of \( \text{H}_2\text{O}_2 \) result in iodine **efflux**

Oxidative stress in *Laminaria*:
- Oxidative (respiratory) burst – a defense reaction
- Desiccation, high temperatures, high irradiance and exposure to atmospheric oxidants at low tide
The oxidative burst in *Laminaria*

- A key element in eukaryotic innate immunity
- Triggers in *Laminaria*: bacterial endotoxins (LPS), oligogulurononates (oligoalginate)

Küpper *et al.*, 2001: Plant Physiology **125**, 278-91
Monitoring the iodine pool during the oxidative burst in *Laminaria* with XAS

- EXAFS: Oxidative stress results in a change of the solution environment of accumulated iodide (towards an aqueous, hydrated form)
- XANES: No changes in the redox state of iodine – only iodide is detectable
Cathodic stripping square wave voltammetry (CSSWV)

- Strong iodide efflux upon oxidative stress
  - No increased levels of oxidized or organic iodine species
Scavenging of ozone (O$_3$) by *Laminaria*

Flow reactor scheme

(Palmer et al., 2005: Env. Chem. 2, 282-90)
Scavenging of ozone (O₃) by *Laminaria*

- *Laminaria* thalli effectively scavenge ozone.

- When light is present: ultrafine particle formation.
Kelp forests contribute to aerosol formation in the coastal environment

- Iodine oxides as condensation nuclei
- “Particle bursts” in the coastal atmosphere above kelp beds at low tide and high irradiance

O’Dowd et al., 2002: Nature 417, 632-6
Kelp forests contribute to aerosol formation in the coastal environment

O’Dowd et al., 2002: Nature 417, 632-6
Kelp iodine emissions into the coastal atmosphere

- “Iodovolatilisation” discovered in 1920s by Kylin and Dangeard: I$_2$ detected with starch paper
- J. Lovelock, 1973: Discovery of methyl iodide emissions from seaweeds
- B. Alicke et al., 1999: High IO levels above kelp beds at low tide
- L.J. Carpenter et al., 2000: CH$_2$I$_2$ main species emitted by Laminaria (total iodine emissions: 0.09 – 0.5 pmol g FW$^{-1}$ min$^{-1}$)
- This study: High I$_2$ fluxes due to reaction of O$_3$ with I$^-$ on seaweed surface (130 pmol g FW$^{-1}$ min$^{-1}$)
You can smell it!

Taynish National Nature Reserve, Argyll, Scotland, June 8, 2008
Sea water: High tide

Atmosphere: Low tide

Particle formation

IO•

O3

Iodine uptake
V-haloperoxidase

H2O2 / ROS

XAS
Biological significance: Iodine accumulation in *Laminaria*

- *Laminaria* accumulates iodide as an inorganic antioxidant: *the first case in a living system!*

- Other, previous hypotheses: chemical defense (grazers, pathogens)

- Implications for atmospheric & marine chemistry
Biological significance: Iodine accumulation in *Laminaria*

- Iodo(hydro)carbons: quantitatively insignificant as H$_2$O$_2$ scavenging products
- Must have another function – defense?!
- Iodine is a better leaving group than bromine or chlorine:  
  I > Br > Cl  

=> High reactivity / strong alkylating potential of iodinated compounds
What makes iodide a suitable antioxidant?

- Reactions with major oxidants ($\text{H}_2\text{O}_2$, $\text{O}_2^-$, $\text{OH}^-$, $^{1}\text{O}_2$, $\text{O}_3$) are very favourable kinetically and thermodynamically
- Iodide compares well with established, organic biological antioxidants
- Iodide is the only halide with potential antioxidant properties
- Confirmed in a heterologous system: iodide quenches respiratory burst in human blood ($\text{IC}_{50} = 2.9 \text{ mM}$)
Complementarity to other antioxidant systems

- Iodide is an **apoplastic** antioxidant (i.e. limited to the cell wall space)
- Ascorbate and glutathione are strictly intracellular (not excreted / effluxed)
- EST data suggest that *Laminaria* has fewer and lower expression levels of typical antioxidant enzymes than other eukaryotes
Iodine accumulation in *Laminaria*


Iodide accumulation provides kelp with an inorganic antioxidant impacting atmospheric chemistry.

Proceedings of the National Academy of Sciences of the USA 105 (19), 5954-58
A place where you can **smell** the tide level...

*Roscoff, Brittany, France*
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• Algal genomes
Algal genome projects

Diatoms:


• *Phaeodactylum* (Bowler *et al.*, 2008: *Nature* **456**: 239-244)
Algal genome projects

- *Ostreococcus tauri*
- *Ostreococcus lucimarinus*
- *Emiliania huxleyi*
- *Micromonas pusilla*
- *Bathyococcus sp.*
- *etc.*

All algal genomics models so far are unicellular!
Ectocarpus siliculosus

- Filamentous, cosmopolitan brown alga – mostly from temperate seas
- One of the best-studied seaweeds
- The first fully-sequenced multicellular alga!
- > 300 fully-characterized strains in public-domain culture collections (CCAP, KU-MACC)

The *Ectocarpus* genome and the independent evolution of multicellularity in the brown algae.- *Nature* 465, 617-21

June 3, 2010
Features of the *Ectocarpus* genome

- 214 Mbp
- Very high number of introns
- Repeated sequences (Transposons, retrotransposons, helitrons) make up > 22% of the genome!
- No ferritins!!
- Halogen metabolism (1 V haloperoxidase, 21 putative dehalogenases and 2 haloalkane dehalogenases)
- Many bacterial genes
- … and a high percentage of genes of still unknown function!
Iodine in *Ectocarpus*?

- *Ectocarpus* accumulates iodine, but at much lower levels than *Laminaria* (data from Roscoff & Vernaison)
- Genome annotation has revealed the presence of one vanadium haloperoxidase gene
- X-ray absorption spectroscopy using synchrotron radiation (XAS) on *Ectocarpus*
Iodine K-edge XAS of *Ectocarpus siliculosus*

- **Ectocarpus in regular Provasoli-enriched sea water, without supplements**
- **Ectocarpus in Provasoli-enriched sea water, with 50 μM iodide supplement**
- **Ectocarpus in Provasoli-enriched sea water, with 50 μM iodate supplement**

➢ **Like Laminaria, Ectocarpus accumulates iodine as iodide!**
Ectocarpus and iron:

axenic cultures are CAS active!

CAS = Chrome azurol S (a colorimetric assay for Fe$^{3+}$-binding activity)

- CAS activity is caused by secretion of strong Fe(III)-binding ligands
  – or acidification
**Siderophores in *Ectocarpus***?

- Siderophore production is known to occur in bacteria, fungi and monocotyledons – but no eukaryotic algae so far! (Only phytosiderophores from monocots / higher plants)

- CAS activity in axenic *Ectocarpus* cultures seemed like an exciting finding

- Still, no siderophores could be isolated so far

- CAS activity might be caused by external acidification
Features of the *Ectocarpus* genome from the bioinorganic perspective: Iron uptake

- homologs of *fro2*, a cell surface Fe(III) reductase
- several divalent metal ABC transporters (ZIP type)
- NRAMP (*M^{2+}-H^{+}* symporter with preference for Fe(II))
  ⇒ consistent with simple reductase/permease pathway
- absence of multicopper oxidases
- presence of siderophore biosynthetic pathway initially hypothesized, but not corroborated
Iron uptake in *Ectocarpus*

- Short term iron uptake studies: Fe is taken up in a time and concentration dependent manner => Consistent with an active transport process.
- Derived kinetic parameters: similar to those reported in the few available studies in other red, green and brown algae

<table>
<thead>
<tr>
<th></th>
<th><em>Macrocystis</em></th>
<th><em>Gracilaria</em></th>
<th><em>Laminaria</em></th>
<th><em>Undaria</em></th>
<th><em>Ectocarpus</em></th>
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</thead>
<tbody>
<tr>
<td>$V_m$</td>
<td>1.6 pmol/cm$^2$/hr</td>
<td>0.26 pmol/mg/hr</td>
<td>2.7 pmol/cm$^2$/hr</td>
<td>6.4 pmol/cm$^2$/hr</td>
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<tr>
<td>$K_m$</td>
<td>3.5 µM</td>
<td>0.6 µM</td>
<td>0.54 µM</td>
<td>6.4 µM</td>
<td>1.5 µM</td>
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<tr>
<td>Ref</td>
<td>Manley 1981</td>
<td>Liu 2000</td>
<td>Matsunaga 1991</td>
<td>Matsunaga 1991</td>
<td>This work</td>
</tr>
</tbody>
</table>
Fe(III) chelate reductase activity in *Ectocarpus*

A) iron replete (30 µM) and B) iron starved (4 nM) cultures of *Ectocarpus siliculosus.*

Negative controls: C) dead cells and D) live cells minus FZ.

Error bars = ± 1 S.D. from triplicate measurements.
Iron uptake from $^{55}$FeEDTA in *Ectocarpus siliculosus* cultures over 800 hrs

Error bars represent ± 1 S.D. from three separate experiments with replicate time points for each.
Features of the *Ectocarpus* genome from the bioinorganic perspective:

Iron storage

- no ferritins!!

⇒ typical feature of heterokont organisms

- No other iron store obvious from the *Ectocarpus* genome

⇒ need for physical techniques
Ectocarpus and iron: How to store iron without ferritin?

- probing the intracellular Fe pool by Mössbauer spectroscopy

...in search of a novel mode of intracellular iron storage...
Ectocarpus and iron:

How to store iron without ferritin?

• probing the intracellular Fe pool by XAS

Ectocarpus and iron:

How to store iron without ferritin?

Mössbauer and X-ray absorption spectroscopy show 2 main components of the cellular Fe pool:

(1) an iron-sulfur cluster (approx. 26% of the total intracellular iron pool)

(2) a second component with spectra typical of a $(\text{Fe}^{3+}\text{O}_6)$ system with parameters similar to the amorphous phosphorus-rich mineral core of bacterial and plant ferritins (approx. 74% of the cellular iron pool)

=> suggests that Ectocarpus contains a non-ferritin but mineral-based iron storage pool
Exotic bioelements in *Ectocarpus*?

- No Cd carboanhydrases (homologous to those in centric diatoms) identified in genome

- However, *Ectocarpus* cell walls accumulate high levels of strontium (Sr)! (Detected by X-ray microprobe)

- Sr is an earth alkali metal – from the 2nd main group (beneath calcium)

- Hypothesis: Probably bound to alginate

- No biological functions of Sr are known
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Thank you.

Dunstaffnage Marine Laboratory, Oban, Argyll / West Highlands, Scotland
The following is reserve material for self study
Reserve slides for the *Laminaria*-iodine section follow
What makes iodide a suitable antioxidant?

**Thermodynamics**

<table>
<thead>
<tr>
<th>One-electron transfer per $X^-$</th>
<th>$\Delta G_R$ (kJ mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$X^- + 3O_2 \rightarrow [X] + O_2^{\cdot -}$</td>
<td>247.97, 200.7, 143.76</td>
</tr>
<tr>
<td>$X^- + \text{OH}\cdot (g) \rightarrow [X] + \text{OH}^-$</td>
<td>49.21, 1.93, -55.0</td>
</tr>
<tr>
<td>$X^- + \text{OH}\cdot(aq) \rightarrow [X] + \text{OH}$</td>
<td>67.54, 20.26, -36.66</td>
</tr>
<tr>
<td>$X^- + 1O_2 \rightarrow [X] + O_2^{\cdot -}$</td>
<td>152.45, 105.17, 48.24</td>
</tr>
<tr>
<td>$X^- + \text{H}_2\text{O}_2 \rightarrow [X] + \text{OH}\cdot + \text{OH}^-$</td>
<td>235.42, 188.15, 131.22</td>
</tr>
<tr>
<td>$X^- + \text{H}^+ + \text{HO}_2\cdot \rightarrow [X] + \text{H}_2\text{O}_2$</td>
<td>93.59, 46.31, -10.61</td>
</tr>
<tr>
<td>$X^- + \text{O}_3 \rightarrow [X] + \text{O}_3^{\cdot -}$</td>
<td>135.08, 87.80, 30.87</td>
</tr>
</tbody>
</table>
What makes iodide a suitable antioxidant?

**Thermodynamics**

<table>
<thead>
<tr>
<th>Two-electron transfer per X^-</th>
<th>Cl^-</th>
<th>Br^-</th>
<th>I^-</th>
<th>ΔG_R (kJ mol^-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 X^- + H^+ + O_3 → X_2 + O_2 + OH^-</td>
<td>-57.9</td>
<td>-112.5</td>
<td>-217.3</td>
<td></td>
</tr>
<tr>
<td>X^- + H^+ + O_3 → HOX + O_2</td>
<td>-111.8</td>
<td>-141.4</td>
<td>-210.8</td>
<td></td>
</tr>
<tr>
<td>X^- + H^+ + 1O_2 + H_2O → HOX + H_2O_2</td>
<td>62.48</td>
<td>32.88</td>
<td>-36.43</td>
<td></td>
</tr>
<tr>
<td>X^- + 2H^+ + 1O_2 → X_2 + H_2O_2</td>
<td>96.66</td>
<td>42.06</td>
<td>-62.76</td>
<td></td>
</tr>
<tr>
<td>X^- + H_2O_2 → HOX + OH^-</td>
<td>28.21</td>
<td>-1.39</td>
<td>-70.81</td>
<td></td>
</tr>
<tr>
<td>X^- + 2 H^+ + H_2O_2 → X_2 + 2 H_2O</td>
<td>-77.66</td>
<td>-132.26</td>
<td>-237.08</td>
<td></td>
</tr>
</tbody>
</table>
What makes iodide a suitable antioxidant?

**Kinetics**

<table>
<thead>
<tr>
<th>Compound</th>
<th>$k_{12}$ (M$^{-1}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>O$_3$ reactions with</strong></td>
<td></td>
</tr>
<tr>
<td>I$^-$</td>
<td>$1.2 \times 10^9$</td>
</tr>
<tr>
<td>Br$^-$</td>
<td>$2.48 \times 10^2$</td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>$&lt; 3 \times 10^{-3}$</td>
</tr>
<tr>
<td>Ascorbate</td>
<td>$4.8 \times 10^7$</td>
</tr>
<tr>
<td>Glutathione</td>
<td>$2.5 \times 10^6$</td>
</tr>
<tr>
<td><strong>Singlet oxygen ($^{1}O_2$) reactions with</strong></td>
<td></td>
</tr>
<tr>
<td>I$^-$</td>
<td>$1 \times 10^{8}$ (aprotic solvents)</td>
</tr>
<tr>
<td></td>
<td>$8.7 \times 10^{5}$ (pH ~ 7)</td>
</tr>
<tr>
<td>Br$^-$</td>
<td>$1.0 \times 10^3$</td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>$1.0 \times 10^3$</td>
</tr>
<tr>
<td>Ascorbate</td>
<td>$8.3 \times 10^6$</td>
</tr>
<tr>
<td>Glutathione</td>
<td>$2.4 \times 10^6$ (in D$_2$O, 310 K, pD = 7.4)</td>
</tr>
</tbody>
</table>
What makes iodide a suitable antioxidant?

**Kinetics**

<table>
<thead>
<tr>
<th>Compound</th>
<th>$k_{12} \text{ (M}^{-1} \text{ s}^{-1})$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OH radical ($\cdot$OH) reactions with</strong> I$^-$</td>
<td>1.2 x $10^{10}$</td>
</tr>
<tr>
<td>Ascorbate</td>
<td>1.1 x $10^{10}$</td>
</tr>
<tr>
<td>Glutathione</td>
<td>1.3 x $10^{10}$ (pH = 5.5)</td>
</tr>
<tr>
<td>Dimethyl sulfoniopropionate</td>
<td>3 x $10^8$</td>
</tr>
<tr>
<td>Dimethyl sulfide</td>
<td>1.9 x $10^{10}$</td>
</tr>
<tr>
<td>Dimethyl sulfoxide</td>
<td>6.6 x $10^9$</td>
</tr>
<tr>
<td><strong>Superoxide (O$_2^-$) reactions with</strong> I$_3^-$</td>
<td>1 x $10^8$ (no data available for I$^-$)</td>
</tr>
<tr>
<td>Ascorbate</td>
<td>2.7 x $10^5$ (pH= 7.4)</td>
</tr>
<tr>
<td>Glutathione</td>
<td>2.4 x $10^5$ (pH= 7.8)</td>
</tr>
</tbody>
</table>
What makes iodide a suitable antioxidant?

**Kinetics**

<table>
<thead>
<tr>
<th>Compound</th>
<th>$k_{12}$ (M$^{-1}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen peroxide ($\text{H}_2\text{O}_2$) reactions with</td>
<td></td>
</tr>
<tr>
<td>I$^-$</td>
<td>0.69</td>
</tr>
<tr>
<td>Br$^-$</td>
<td>$2.3 \times 10^{-5}$</td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>$1.1 \times 10^{-7}$</td>
</tr>
<tr>
<td>Ascorbate</td>
<td>$2 \times 10^{0}$</td>
</tr>
<tr>
<td>Glutathione</td>
<td>$2 - 20 \times 10^{0}$</td>
</tr>
<tr>
<td>Glutathione peroxidase</td>
<td>$6 \times 10^{7}$</td>
</tr>
</tbody>
</table>