Iron: Mechanisms of Pro-oxidant Behavior, Cellular Uptake, and Organism Survival Skills

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Electron transport and cellular respiration e.g. cytochrome C in the mitochondria

Cellular detoxification of free radicals by peroxidases and catalases in peroxisomes and lysosomes

Cellular proliferation e.g. DNA synthesis involving ribonucleotide reductase

Post translation regulation of gene expression involving iron regulatory proteins
Haber-Weiss Reaction

\[ \text{O}_2^- + \text{Fe}^{3+} \rightarrow \text{O}_2 + \text{Fe}^{2+} \]

\[ \text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \text{HO}^- \]

\[ \text{O}_2^- + \text{H}_2\text{O}_2 \rightarrow \text{O}_2 + \text{OH}^- + \text{HO}^- \]
Reaction of Iron With Lipid Hydroperoxides

\[ \text{Fe}^{2+} + \text{ROOH} \rightarrow \text{Fe}^{3+} + \text{OH}^- + \text{RO}^\bullet \]
Iron Can Contribute Directly or Indirectly to the Oxidation of:

- Proteins
- Lipids
- DNA
- Sugars
- Site-specific Oxidation?
Human Iron Metabolism

- Iron exists in $2^+$ (ferrous) or $3^+$ (ferric) state
- Little Free Iron *in vivo*
- Chelated to Proteins or Other Molecules
  - Maintains Solubility
  - Limits Participation in Oxygen Redox Chemistry
  - Limits Availability to Microbes
- Iron-Binding Proteins Vary With Location
Extracellular Iron Chelates

- Transferrin
  - Serum
  - Mucosa (e.g. lung)
- Lactoferrin
  - Mucosa (e.g. lung)
  - Milk
  - Neutrophils
Transferrin and Lactoferrin

- 80 kDa glycoproteins
- Bind Ferric Iron With High Affinity
- Two Iron-Binding Sites per Molecule
- Enhanced by the presence of anions – e.g. carbonate
- Binding is pH sensitive
- Lactoferrin better iron retention at low pH
Lactoferrin

Biochemistry 31:4527-33, 1992
Iron Bound To Transferrin Or Lactoferrin Does Not Redox Cycle

Unfavorable reduction potential

<table>
<thead>
<tr>
<th>Reaction</th>
<th>$E^\circ$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe(III) Transferrin/Fe(II) Transferrin</td>
<td>-400 mV</td>
</tr>
<tr>
<td>Fe(III) Ferritin, 2H$^+$/Fe(II) Ferritin</td>
<td>-190 mV</td>
</tr>
<tr>
<td>Fe(III) EDTA/Fe(II)/EDTA</td>
<td>+120 mV</td>
</tr>
<tr>
<td>Fe(III) Citrate/Fe(II) Citrate</td>
<td>+≈100 mV</td>
</tr>
<tr>
<td>Fe(III)ADP/Fe(II) ADP</td>
<td>+≈100 mV</td>
</tr>
<tr>
<td>$O_2/O_2^\cdot$</td>
<td>-330 mV</td>
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Haber-Weiss Reaction

\[ \text{O}_2 \cdot^- + \text{Fe}^{3+} \rightarrow \text{O}_2 + \text{Fe}^{2+} \]

\[ \text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \text{HO} \cdot \]

Lactoferrin or Transferrin
Intracellular Iron Chelates

• Ferritin
  • Long term storage
  • 4500 atoms Fe/molecule
  • Fe$^{3+}$

• Labile Iron Pool
  • Poorly characterized
  • Transient storage
  • Exchanges with ferritin
Iron Bound To Ferritin Is Also Relatively Non-Reactive

Unfavorable reduction potential

\[ E^\circ \]

Fe(III) Transferrin/Fe(II) Transferrin - 400 mV
Fe(III) Ferritin, 2H⁺/Fe(II) Ferritin - 190 mV
Fe(III) EDTA/Fe(II)/EDTA + 120 mV
Fe(III) Citrate/Fe(II) Citrate +\(\approx\)100 mV
Fe(III)ADP/Fe(II) ADP +\(\approx\)100 mV
\(O_2/O_2^{\cdot-}\) - 330 mV
HOW IS IRON TRANSPORTED INTO CELLS?
Receptor-Mediated Iron Uptake From Transferrin
Transferrin Receptor Complex
HFE Protein Interacts With The TFR

Normal  Hemochromatosis

Blood 92:1845-51, 1998
TFR2

- Newly described receptor for transferrin
- Liver and peripheral blood mononuclear cells
- Lower affinity for transferrin than TFR1
- About 60% sequence homology to TFR1
- Doesn’t bind HFE
- Mutations of TFR2 are associated with hemochromatosis
Fe Uptake From Lactoferrin

- Binding to Variably Characterized Surface “Receptors”
  Not TFR

  Proposed Receptors
  Protein; Glycosaminoglycans; Scavenger Receptor; Asialoglycoprotein Receptor; Mannose Receptor

- No Agreement on Cellular Fe Acquisition from LF

- ? Fe Handled Differently than when Acquired from TF
What’s Known About Fe Uptake From LMW Chelates

- Most cell types can do so
- Variable ill-defined mechanisms involved
- Inducible in myeloid cells
  - Multivalent metals
  - ATP independent
  - Not receptor-mediated endocytosis
Gallium Induces Fe Uptake From LMW By HL-60 Cells

Ascorbate = NTA > ADP > citrate >> NTA (No Ga)

Pathway for High Affinity Iron Uptake in Yeast

## Iron Transporters

### Yeast vs. Mammalian Cells

<table>
<thead>
<tr>
<th>Function</th>
<th>Yeast cells</th>
<th>Mammalian cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferrireduction</td>
<td>FRE1 and FRE2</td>
<td>Gp91 phos-related protein?</td>
</tr>
<tr>
<td>Divalent metal ion</td>
<td>SMF1, other SMF</td>
<td>DMT1/Nramp2, Nramp1 and other family members?</td>
</tr>
<tr>
<td></td>
<td>family members</td>
<td></td>
</tr>
<tr>
<td>Fe(II) uptake</td>
<td>FET4</td>
<td>Not known</td>
</tr>
<tr>
<td>FE(III) uptake</td>
<td>FTR1</td>
<td>Not known, MIP pathway?</td>
</tr>
<tr>
<td>Ferroxidation</td>
<td>FET3</td>
<td>Ceruloplasmin, hephaestin</td>
</tr>
<tr>
<td>Fe export</td>
<td>Not known</td>
<td>Ireg1/Ferroportin/MTP1</td>
</tr>
</tbody>
</table>

HOW IS INTRACELLULAR AND EXTRACELLULAR IRON CONTENT REGULATED?
Structure of the Consensus Iron Responsive Element

Six-membered loop, the first five bases of which are almost always CAGUG. The sixth base is most often a pyrimidine.

Bulge that is invariably a cytosine residue.

"Upper" stem usually consists of five base pairs. NN represents any complimentary pair of RNA bases.

"Lower" stem of variable length. TFR IREs are AU-rich here. Ferritin IREs have more GC content, but also have additional unpaired bases.

The Interaction of IRP-1 with Ferritin and Transferrin Receptor mRNA

Iron Control of Translation and mRNA Stability

- Ferritin mRNA
  - High intracellular iron: IRP1
  - Low intracellular iron: IRP1
  - IRP1 blocks translation, decreases ferritin levels
  - IRP1 protects mRNA, increases TFR levels

- TFR mRNA
  - No protection of mRNA, rapid degradation, decreases TFR levels

Int Rev Cytol 211: 241-278, 2001
Heme Oxygenase and Iron Metabolism

Iron Metabolism and Host Defense

• Nearly Every Microorganism Needs Iron for Growth and Metabolism
  • Enzymes
    • DNA replication
    • Respiratory chain
    • Antioxidants
    • Heme centers

• Iron Bound to Lactoferrin and Transferrin is Much Less Accessible
Infection Shifts Iron

- Host Response to Acute or Chronic Infection
  - Shift Iron Out of Serum
  - Shift Iron Into Reticuloendothelial System
    Macrophages
- Good Against Extracellular Pathogens
- Perhaps Not So For Intracellular Ones
How Do Pathogens Acquire Iron From the Host?
Fe Sources Potentially Available To Pathogens

Microbial Strategies of Iron Acquisition from Extracellular Host Iron Chelates
Siderophore-Mediated Iron Uptake

Ferri-siderophore Transport in Gram-negative Bacteria

Uptake of Transferrin Iron by Gram-negative Bacteria

Other Microbial Pathogens

- Fungi
  - Siderophores
  - Fe reduction
- Protozoan Parasites
  - Trypanosomes – TF receptor
  - Leishmania – TF or LF Receptor?
  - Trichomonas – TF or LF receptor
  - Malaria
Gene Regulation by the Fur Protein
Table 3. Occurrence of the Fur protein in Gram-negative pathogenic bacteria and of related functional proteins (DtxR and IdeR) in Gram-positive bacteria

<table>
<thead>
<tr>
<th>Fur</th>
<th>DtxR/ IdeR</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus subtilis</em></td>
<td><em>Brevibacterium lactofermentum</em></td>
</tr>
<tr>
<td><em>Bordetella</em> spp.</td>
<td><em>Corynebacterium glutamicum</em></td>
</tr>
<tr>
<td><em>Campylobacter jejuni</em></td>
<td><em>Corynebacterium diphtheriae</em></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td><em>Mycobacterium spp.</em></td>
</tr>
<tr>
<td><em>Haemophilus influenzae</em></td>
<td></td>
</tr>
<tr>
<td><em>Neisseria</em> spp.</td>
<td></td>
</tr>
</tbody>
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*Ann Rev Microbiol. 54: 881-941, 2000*
Fe Sources Potentially Available To Pathogens

Iron Uptake and Trafficking in M. tuberculosis-infected Macrophages

FeTf = Fe-transferrin (diferric transferrin)
TfR = transferrin receptor
SMW = small molecular weight
Mycobacterial Iron Acquisition

Siderophores (low MW Fe chelators)
- Mycobactins - hydrophobic siderophores associated with the bacterial membrane
- Exochelins - water soluble, secreted siderophores
  - Peptidohydroxamate type (*M. smegmatis*)
  - Carboxymycobactin type (*M. tb*, MAC)
Iron Uptake and Trafficking in M. tuberculosis-infected Macrophages

FeTf = Fe-transferin (diferric transferrin)
TfR = transferrin receptor
SMW = small molecular weight
M.tb Fe Uptake Decreases in MDM From Patients With Hereditary Hemochromatosis

**M.tb**

- Exogenous, 24 hrs
- Endogenous, 24 hrs

**MDM**

- Exogenous, 24 hrs
- Endogenous, 24 hrs

M.tb-associated Fe (pmol)

MDM-associated Fe (nmol)
Bacterial Iron Storage

- Bacterioferritin
- Bacterial Ferritin
- Labile Iron Pool
- Mechanisms poorly defined
- Aconitase as a source of increased redox active iron
THE END
Reviews and Selected Original Articles


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