Hemochromatosis & Ferritin

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Hemochromatosis

• The most common form of iron overload disease
Causes

- Mainly by mutations in a gene called HFE
- Two major and most common mutations C282Y and H63D

Those who inherit defective gene from only one parent usually do not develop disease but might have higher than normal iron absorption levels.
Types

• Primary: Inherited.
• Secondary: Caused by something else.

Symptoms

• Cardiomyopathy: Heart abnormalities
• Cirrhosis of the Liver
• Impotence
• Arthritis
• Early menopause
• Abnormal skin pigmentation
• Thyroid deficiency
• Adrenal gland damage
Why should we care?

• 1 to 2 in 200 people in the United States carry both copies of the defective gene and can develop the disease.

• Can be a precursor for more serious/fatal diseases.
Diagnosis

• **Special blood test**
  screen for HFE mutations

• **Serum ferritin levels**
  Normal levels
  Adult males: 75-175 micrograms/dL
  Adult females: 65-165 micrograms/dL
  Children: 50-120 micrograms/dL
  Newborns: 100-250 micrograms/dL

• **Transferrin saturation levels**
  Normal Levels
  Serum iron: 60-170 mcg/dl (10-30umol/L)
  TIBC: 240-450 mcg/dl
  Transferrin saturation: 15-50% (males), 12-45% (females)

• **Liver Biopsy**
Treatment

• Phlebotomy, removes blood the same way it is drawn from donors at blood banks.

• Based on severity, a pint is taken once or twice a week for several months to a year.

• Another option is to undergo chelation therapy, which uses chemical compounds to remove iron from the blood.
Iron (Fe)

- Primary source of Iron: Food we eat
- Iron has two oxidation states (Fe$^{+2}$, Fe$^{+3}$)
- It is ingested in the +3 state and reduced to +2 once in the stomach
- Free iron is toxic to cells; It acts as catalyst for the formation of free radicals via Fenton chemistry
Free Radical Chemistry

(Fenton-Reaction)

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

Highly reactive

Free radicals such as \((\text{OH}^-)\) are known to attack and damage cells and tissue
Iron is an element where you
“Can’t live without it, Can’t live with too much of it”

Dual Paradox of Iron

Useful but hazardous

Abundant but poorly available

Therefore the need of means to store iron in a bio-available form

FERRITIN
FERRTIN
Iron deficiency (The world greatest nutritional problem)

Iron Overload (Hemochromatosis)

30% of the world suffer from iron deficiency
(Sickle Cell anemia, Thalassemia)

Iron is needed for several metabolic reactions in the body
Ferritin

- Function: Iron storage
- MW: ~ 500,000 Da
- 24 subunits (channels)
- Structure: Shell
- Iron capacity: 4500 Fe$^{3+}$ per molecule
- Ferrooxidase activity: Fe$^{2+} \rightarrow$ Fe$^{3+}$ + e$^{-}$
Iron stored as a mineral inside ferritin

Ferritin controls the amount of available iron in the body, preventing iron disorders like anemia and iron overload.
It is important to understand diseases and the mutations that cause them on a molecular level to allow us design more effective treatment options and possible cures.

My Project

The main questions we asked were:

• How is iron taken up and stored by the protein?

• How is it released to cells?

• What pathway does iron take to get inside?

• What are the different steps involved in the iron uptake and release?
My Project
IRON-STORAGE AND DETOXIFICATION PROTEIN

Iron stored as mineral inside ferritin

PROTEIN SUBUNIT
How is Iron taken up?
How is Iron oxidized and stored?

**Ferroxidation Mechanism:**

\[ 2\text{Fe}^{2+} + \text{O}_2 + 4\text{H}_2\text{O} + \text{P} \rightarrow \{ \text{P-}[\text{Fe}_2\text{O}_2]^{2+} \} \rightarrow \text{µ-1,2-peroxodiFe(III)} \]

\[ (\text{~5 min}) \]

\[ \{ \text{P-}[\text{Fe}_2\text{O(OH)}_2]^{2+} \} \rightarrow \text{P + 2FeOOH} \text{(core)} + \text{H}_2\text{O}_2 + 4\text{H}^+ \]

(50 ms)  (10-15 s)

Ferritin molecule

Ferroxidase center of Ferritin
High-Tech SFA-20M Stopped-Flow spectrophotometer
Oximetry

\[
2\text{Fe}^{2+} + \text{O}_2 + 4\text{H}_2\text{O} + \text{P} \rightarrow \{ \text{P-[Fe}_2\text{O(OH)}_2]^{2+} \} + \text{H}_2\text{O}_2 + 2\text{H}^+ 
\]

pH-stat

\[
\text{Net NaOH added (\mu M)}
\]

\[
\text{Time (sec)}
\]

\[
\text{[O]}_2 \text{M}
\]

Graph showing the change in net NaOH added over time with corresponding change in \([O]\_2\) concentration.
Where does Iron Bind?

Recall, Ferritin has 24 subunits.
How does ferritin help you Store Iron safely?

Attenuation of hydroxyl radical chemistry

$$\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \text{OH}^.$$
Effect of Fe Ligand Substitutions

$\frac{1}{2}$ of $O_2$ uptake (sec) vs Fe(II)/protein
Conclusions

- Identified the pathway by which iron is taken up into the protein
- Characterized the iron oxidation mechanism
- Defined the stoichiometric reaction of iron oxidation
- Showed that ferritin is able to attenuate free radical production
- Determined the maximum iron capacity (4500 Fe/protein)
- Additional experimental results were obtained but for lack of time are not shown here
NEW INSIGHTS INTO THE ODD IRON OXIDATION STOICHIOMETRY
AND THE ROLE OF THE C-SITE IN E. COLI BACTERIAL FERRITIN

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Abstract
At least three ferritins are found in the Escherichia coli bacterium, the heme-containing bacterioferritin (EcBFTR) and two non-heme bacterial ferritins (EcFtnA and EcFtnB). In addition to conserved iron ligands at the A and B sites of the ferroxidase center, EcFtnA has a unique third iron binding site (C-site) of unknown function that is ~ 9 Å away from the direct active site. With the help of site-directed mutagenesis, we investigated the role of this third C-site and further defined the mechanism by which EcFtnA oxidizes and stores iron inside its shell. A binding stoichiometry of 48 Fe(III) per EcFtnA is obtained by titration using aximetry, pH stat, fluorescence and UV-315 spectroscopy, in accord with recent isothermal titration calorimetry data. The presence of the C-site in EcFtnA causes the iron oxidation stoichiometry of the first 48 Fe(III)/shell added to increase from ~ 2 to ~ 3 Fe(III)/O2. While the C-site is not required for fast oxidation of the first 48 Fe(III)/shell at the D-iron ferroxidase center of EcFtn3, all C-site variants (E49A, E126A and E130A) exhibit a Fe(III)/O2 stoichiometry of ~ 2:1 similarly to human recombinant H-chain ferritin (HuHFT), demonstrating that the C-site strongly influences the oxygen redox chemistry. The initial rates of Fe(III) oxidation in EcFtnA and all of its variants (except for the C-site variant E49A) decrease markedly after the first 48 Fe(III)/protein have been oxidized. Low temperature EPR spectroscopy suggests the formation of a transient protein radical, possibly involving Tyr24 situated ~ 2.5 Å from the Fe(III) ion at the B-site. The data is discussed in terms of a plausible mechanism that explains the odd iron oxidation stoichiometries in the non-heme bacterial ferritin EcFtnA.

Introduction
The inappropriate control of iron can lead to the generation of damaging oxygen radicals and therefore compromise cellular function. In oxygenated environments, both prokaryotes and eukaryotes have developed highly efficient mechanisms to acquire iron and ensure bioavailability while preventing toxicity (1). Ferritin, a widely distributed intracellular iron storage and detoxification protein consists of two functionally and genetically distinct subunit types, H and L, in mammals. These two subunits co-assemble in various ratios to form a shell-like structure where thousands of iron atoms can be stored within its 8 nm diameter cavity. The H-subunit has a dinuclear iron center consisting of A & B binding sites where the fast conversion of Fe(II) to Fe(III) by dioxygen occurs whereas L-subunit thought to contribute to the modulation of the iron core and thus stores iron at a lower rate compared to the H-subunit (1-5).

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