Iron management and transport

PG Third Semester

Bioinorganic Chemistry-II

Lecture 3 & 4

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Contents

- **Siderophores**
- **Ferritin**
- **Transferrin**

Books/References used and suggested

- Bioinorganic Chemistry by Bertini, Gray, Lippard and Valentine
- Inorganic Biochemistry by Cowan
- Bioinorganic Chemistry by A. K. Das
- Environmental Chemistry by A. K. De
- Oxford Chemistry Primer by Fenton

In-vivo transport and storage of iron

- Fe is essential for life; exists in two forms Fe(II) and Fe(III)
- Two main problems

  (i) Insolubility of Fe(III): At physiological O_2 concentrations, Fe(II) is readily oxidized to Fe(III), which is highly insoluble in aqueous solution at normal pH.

  (ii) Toxicity of free Fe species through the generation of free radicals causing severe cell damage.
- Nature has developed sophisticated chemical system to execute and acquisition to its subsequent transport, storage and utilization in tissue.
- Fe storage system must be able to respond to ‘supply and demand’ (store the excess, and must mobilize & release when needed), as the amount of Fe in the diet is variable.
- Ferritin stores and transferrin transports Fe in mammals.
- Siderophore stores and transports Fe in microorganisms.

Storage of Iron

- Three properties of Fe accounts for its extensive use in biological processes

(i) facile redox reactions of iron ions;

(ii) an extensive range of redox potentials available by ligand substitution

(iii) abundance and availability under conditions apparently existing when terrestrial life began

- The combination of the reactivity of Fe(II) ion and the relatively large amounts of Fe used by cells have necessitated its storage

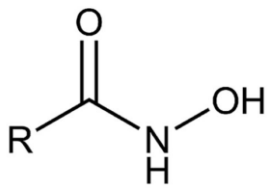
- The transition in the atmosphere (about 2.5 billion years ago) resulted in drop in bioavailability of Fe thereby increasing the need for its storage.

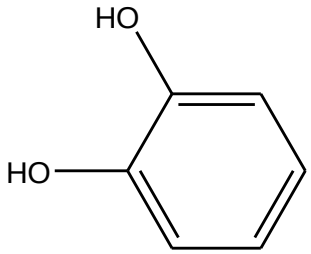
- Comparison of the solubility of Fe^{3+} at physiological conditions (~10^{-18} M) to the Fe content of cells (~ 10^{-5} to 10^{-8} M) emphasizes the difficulty of acquiring sufficient Fe

Siderophores: Fe-storage and transport in lower organisms

- Iron transfer compounds in microorganisms (bacteria and fungi)
- Small polydentate ligand with high affinity for Fe(III)
- They have peptide backbones and are strong chelating agents.
- They sequester Fe to give a soluble complex that re-enters the organism at a specific receptor. Once inside the cell, the Fe is released.
- The first life forms on the earth's surface grew in a reducing atmosphere, in which iron was more available because it was present as Fe(II) compounds (higher solubility).
- In oxidizing environment, microorganisms were forced to deal with the insolubility of Fe(III) hydroxide
- On Fe deficiency a secrete high-affinity iron binding compounds called siderophores comes into play.

- Present in most aerobic microorganisms (bacteria)
- Solubilize and transport iron as Fe(III)



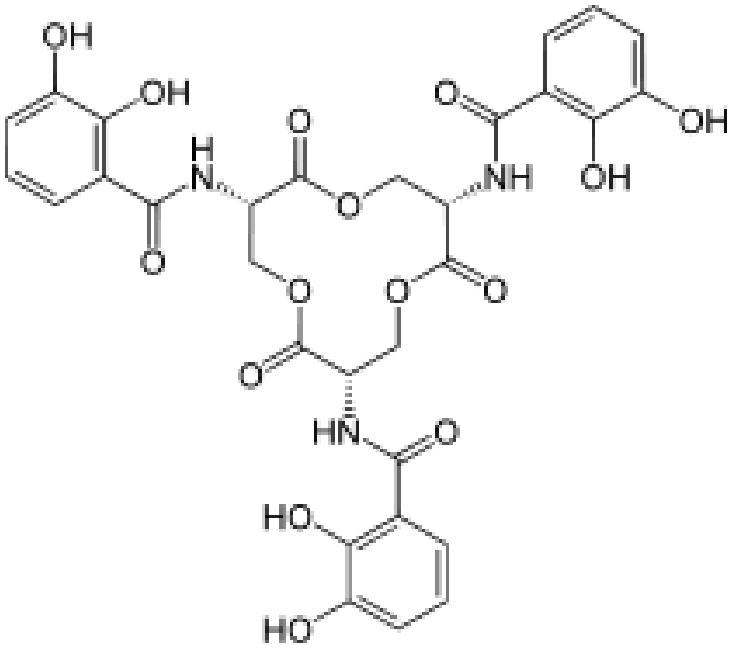


- Low molecular weight compounds (500-1000 D) and have high affinity for iron.
- They can be grouped into two general categories viz. one having hydroxamate ligands (ferrichromes, ferrioxamines) and other having catecholate (o-dihydroxybenzene) ligands (enterobactin).
- These molecules are polydentate ligands with many potential ligating atoms (HSAB principle) to form chelate.
- Readily form extremely stable octahedral complexes with high spin Fe(III).
- Although stable, the complexes are labile (HS, d^5, no CFSE) enough to allow transport and transfer of iron within the bacteria.

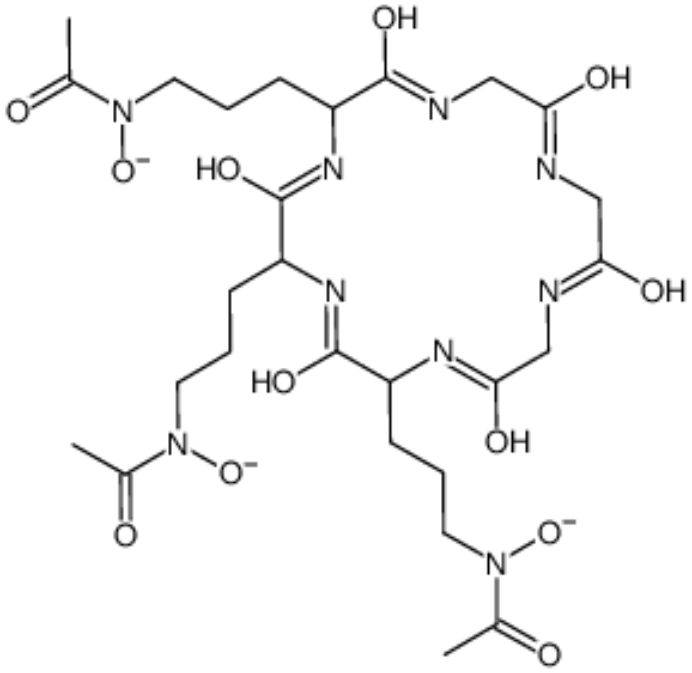
Hydroxamate and catecholate ligands

- Ferrichromes (a cyclic hexapeptide consisting of three glycine and three N- hydroxyl-1-ornithines) and ferrioxamines are trihydroxamic acids which form neutral tris chelates from three bidentate hydroxamate monoanions.
- In enterobactin, each catechol group behaves as a dianion for a total charge of -6 for the ligand (association constant for Fe(III) is approximately 10^{50}).
- In addition to form globular complexes, siderophore molecules consists of a symmetric hydrophilic portion that plays a key role in transport of iron in cell membrane.
- Tris chelated octahedral complexes can provide optical isomer and thereby can be chiral.
- Since the complexes are labile, ligand or metal exchange can be incurred in these complexes. For example, compared of Fe(III), Cr(III) (d3 with significant CFSE) analogues are significantly inert. Similarly, V(IV) although a bit smaller, can replace Fe(III) in enterobactin.
- Other synthetic analogues and treatment of Fe toxicity by desferrioxamine B.

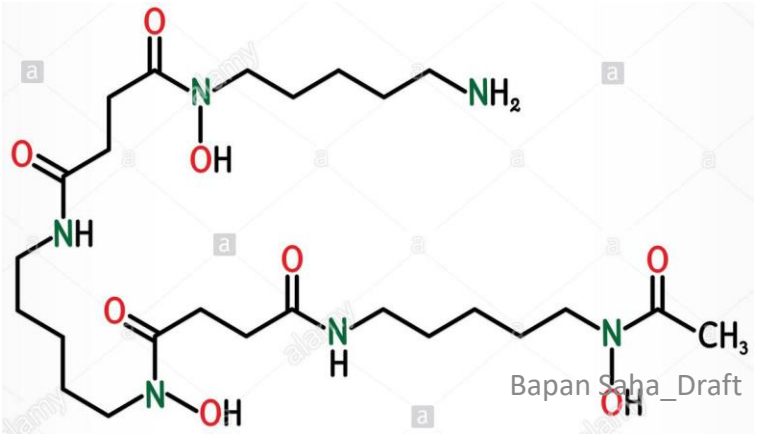
Examples of Siderophores



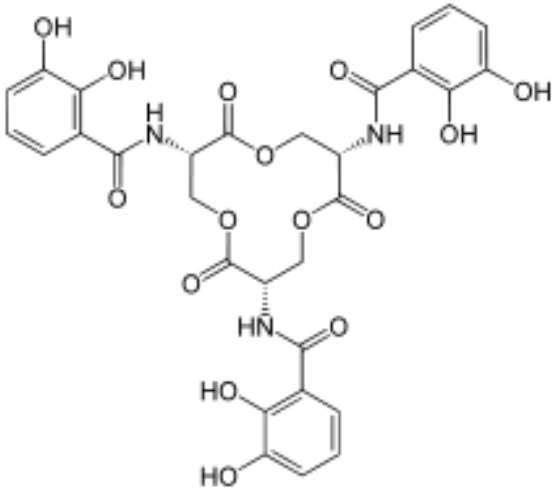
Enterobactin



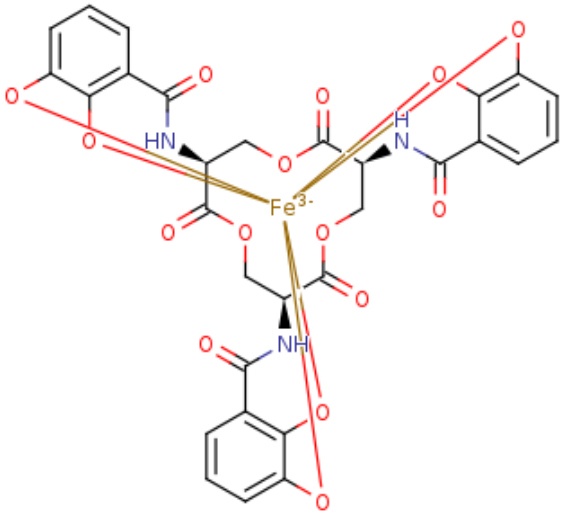
desferrichrome



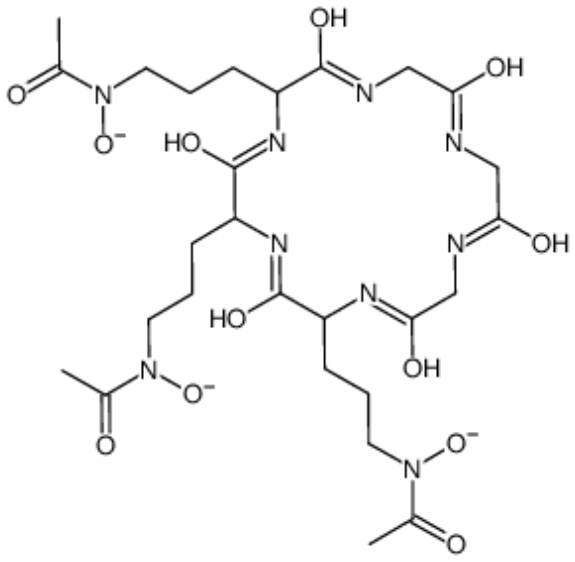
Desferrioxamine B



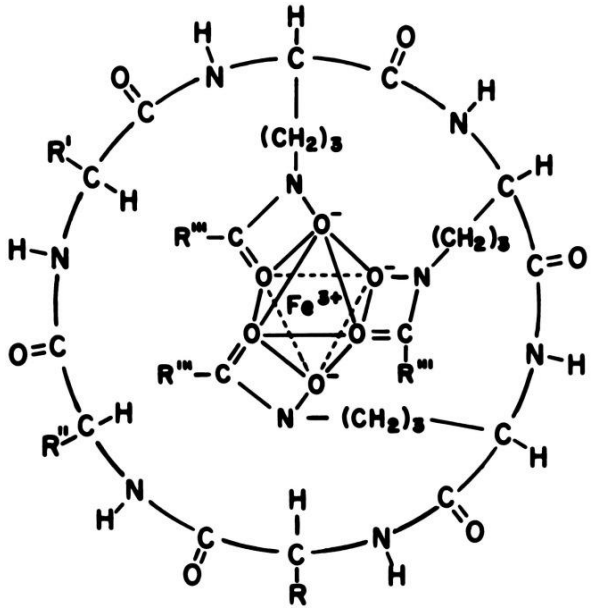
Enterobactin



Fe(III)-enterobactin



ferrichromes

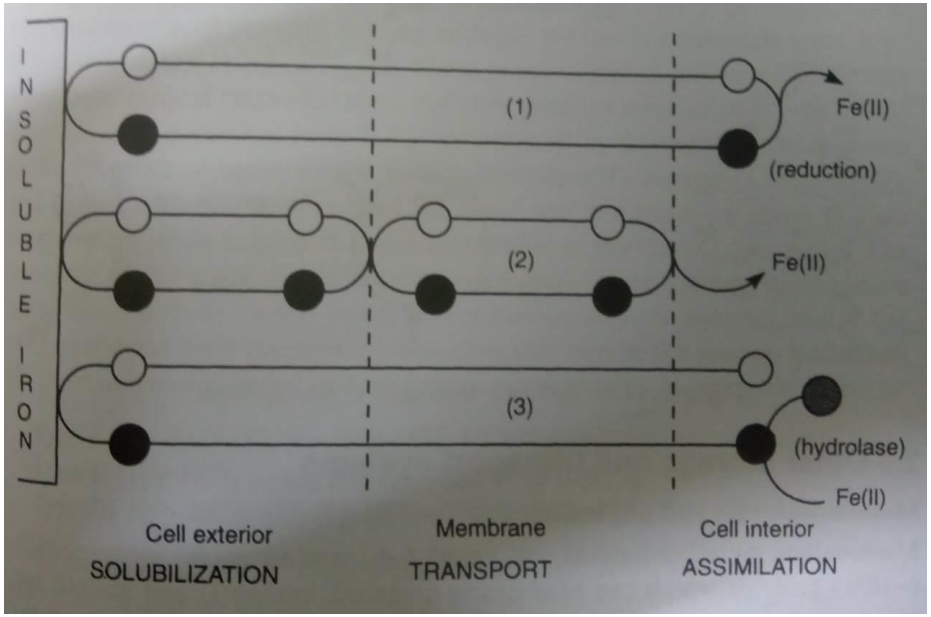


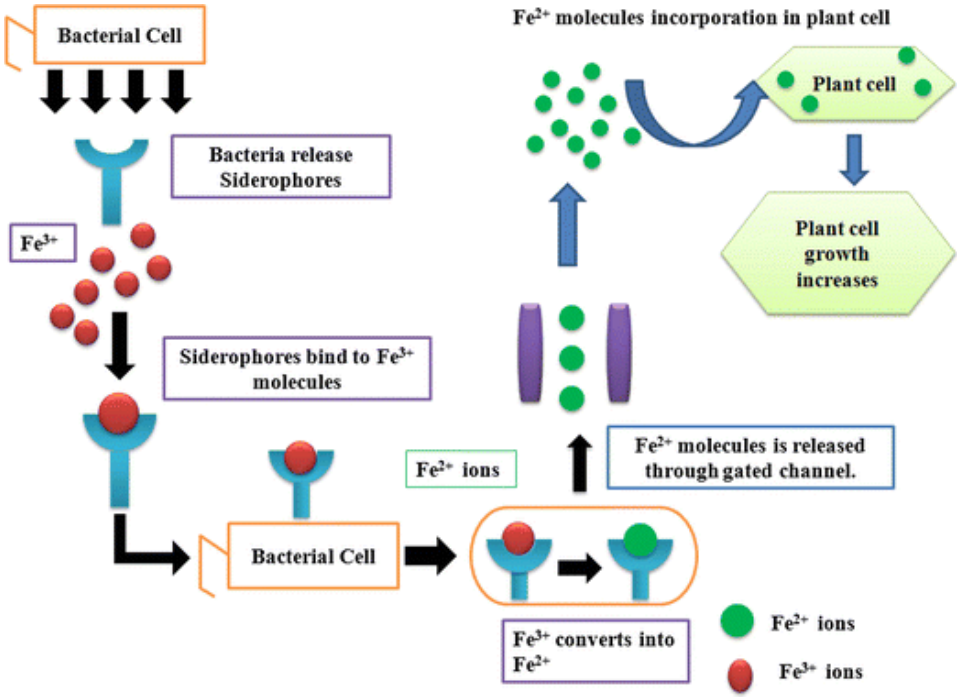
Fe(III)-ferrichromes

Fe uptake mechanism of siderophore

- Three different types of mechanism have been proposed for siderophores activity
- First, the siderophore (ferrichrome) transports the metal across the cell membrane to the cell interior where it is released by a non destructive process and hence ligands are available for reuse.
- Second, the siderophore (ferrioxamine) delivers the metal to the outer cell membrane surface where it is transferred to a secondary transport device which carries it to the cell interior where it is released.
- Third, the metal is transported across the cell membrane by the siderophores (enterobactin) to the cell interior where the complex is broken up, by a hydrolase, so destroying the ligand and giving an example of built in obsolescence
- Enterobactin-mediated Fe uptake in *E. coli* is one of the best characterized of iron uptake processes in microorganisms.

- Ferric enterobactin accumulated in *E. coli*, has to pass through the outer membrane, the periplasm and cytoplasm membrane, and is probably subjected to reduction of the metal in a low pH compartment or to ligand destruction.
- After complexation, the Fe(III)-enterobactin complex interacts with a specific receptor in the outer cell membrane and then taken into the cell by active transport.





Ferritin

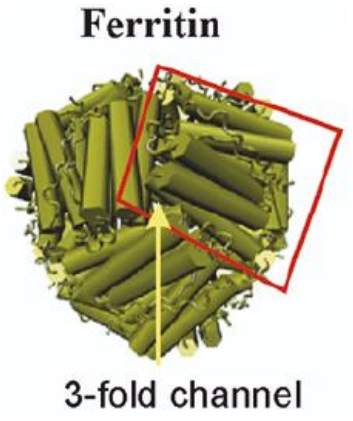
- Ferritin is a storage protein for Fe in non toxic form.
- It is present in liver, spleen and bone marrow, and in plants and bacteria.
- Ferritin is known to release Fe to the developing fetus
- It is consists of an Fe-mineral core (hydrated Fe(III) oxide) surrounded by a protein coat/sheath (apoferritin) with varying amount of phosphate
- The diameter of the core is ~ 80Å and ~ 4500 Fe atoms can be reversibly stored inside the protein coat.
- The lipophilic sheath makes the Fe(III)-complex soluble in biological fluid.
- Apoferritin allows controlled access to the core though eight hydrophilic channels (perhaps Fe(III) enters) and six hydrophobic channels (perhaps Fe(III) leaves)
- Fe(III) ion is of high spin nature and is subjected to strong antiferromagnetic coupling

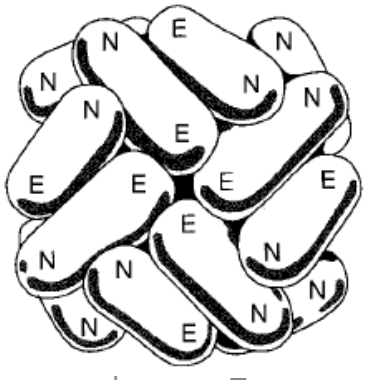
- The role of the stored iron in ferritin varies (intracellular use for biosynthesis of Fe-proteins or mineralization, long term storage and detoxification of excess Fe)
- Iron regulates the synthesis of ferritin, large amounts of ferritin is associated with Fe excess and its small amounts is associated with Fe deficiency.
- Ferritin is also known to be a precursor of several forms of Fe in living organisms (hemosiderin in lysosomes of animals – Fe complex with protein is insoluble).
- Magnetite (Fe_3O_4) is another form of biological Fe derived from the Fe in ferritin. It plays a role in the behavior of magnetic bacteria, bees, and homing pigeons
- The structure of ferritin can be considered to consist of three units: the protein coat, the iron-protein interface, and the Fe core

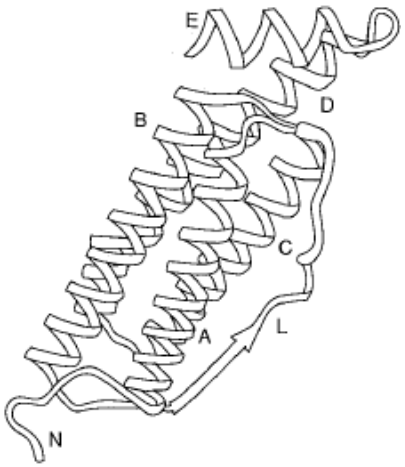
Structure of ferritin

Protein coat/sheath (Apoferritin)

- The protein sheath consists of subunits of 24 polypeptide chains (about 175 amino acid) folded into ellipsoids (lozenge like shape).
- Each subunit is approximately cylindrical & linked together to form a hollow sphere with two-fold, three-fold and four-fold axes.
- The hollow sphere is about 100 Å, the organic surface is about 10 Å thick.
- Two ends of the apoferritin subunit is different and designated as N (the polar N-terminal end of the protein) & designated as E (the non polar helical segment)







- At 8 places, three subunits meet with their N-ends to form a polar channel of three fold symmetry (C_3) through which Fe-can be transferred in or out.
- The three fold polar channels are lined up with hydrophilic aspartate or glutamate residues
- There are 6 non polar channels of C_4 symmetry produced by the meeting of four subunits with their E-ends and are lined up with hydrophobic amino acid residues.
- The protein coat is stable with or without Fe and hence the center of the hollow sphere may be filled with solvent, $Fe_2O_3.H_2O$, or with both small aggregates of Fe & solvent.
- Amino-acid sequences found in ferritin from animals and plants are quite similar.
- Amino acids required to form the shape of the protein coat & the ligands for Fe core is not established (perhaps tyrosine acts as an Fe(III)-ligand)

Iron-Protein interface

- Formation of the iron core is initiated at an Fe-protein interface where Fe(II)-O-Fe(III) dimers and small clusters of Fe(III) atoms have been attached to the protein and bridged to each other by oxo/hydroxo bridges.
- Multiple nucleation sites are available (from electron microscopy)
- Fe is transferred in or out via C_3-symmetry formed by polar N-end of three subunits.
- The coordination of Fe to the protein occurs via carboxyl groups from glutamic (Glu) & aspartic (Asp) acids (from EXAFS - Extended X-ray Absorption Fine Structure & Mossbauer spectra)
- An Fe(III)-tyrosinate (Fe(III)-ligand) complex is present as a transient precursor to polynuclear cluster formation (from by UV-Vis and resonance Raman spectroscopy)

Iron core

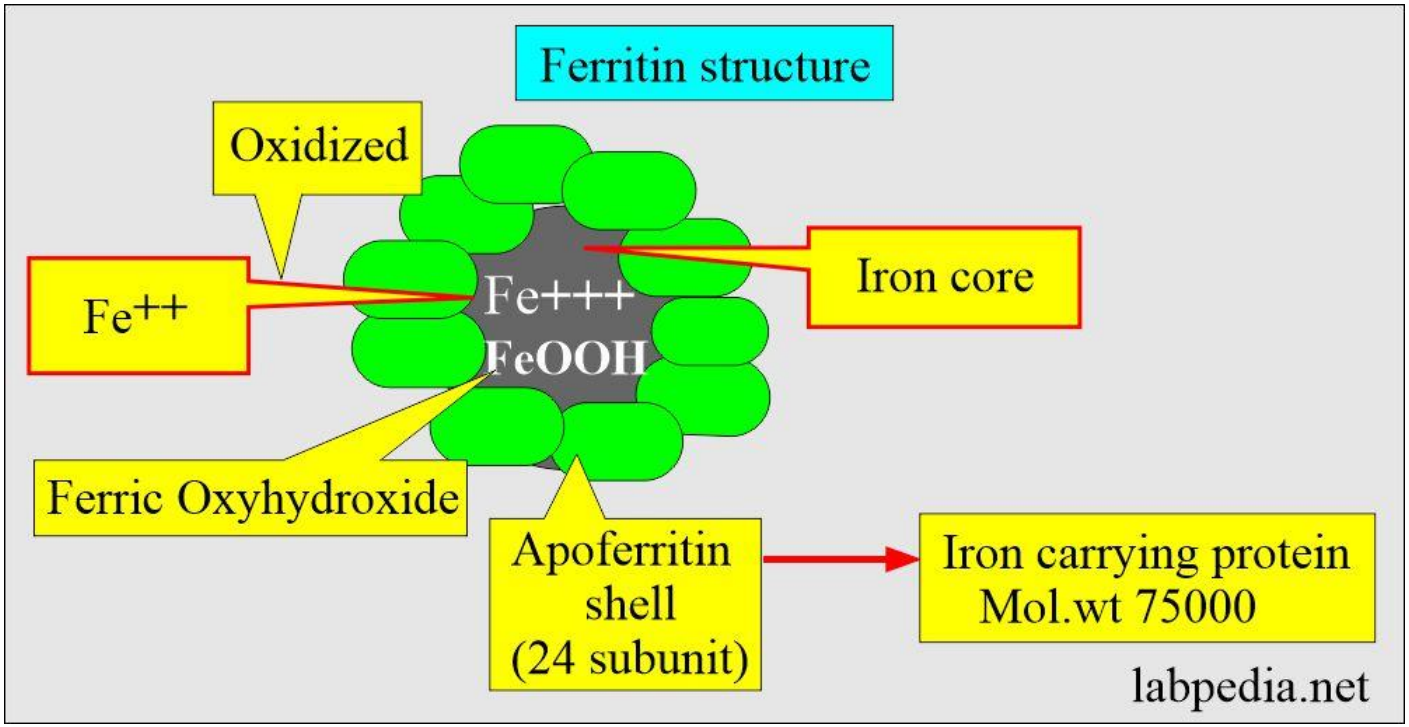
- The core is primarily consists of a sheet structure of Fe(III)-oxide, properties similar to naturally found mineral ferrihydrite ($5Fe_2O_3.9H_2O$)
- The composition of the microcrystalline core is $[(FeOOH)_8(FeO).H_2PO_4]$
- All the Fe-centers are octahedrally surrounded by oxygen
- A hexagonal close packed layers of oxygen (O^{2-} ions) with the randomly distributed layers of octahedrally coordinated Fe(III) atoms (from XRD-data)
- Hydroxide ions (counterbalance the charge) are also present along with oxide ions (from EXAFS - extended X-ray absorption fine structure)
- Fe(III) atom in ferritin is surrounded by six O-atoms at a distance of 1.95 Å and six Fe-atoms at distances of 3.0 to 3.3 Å (from Mossbauer spectroscopy and EXAFS study)
- The phosphate groups act in a terminal roles perhaps covering the (FeOOH) particles and anchoring them to the protein shell (counterbalance the charge)

- All ferritin cores are not the same, some variations in the degree of structural and magnetic ordering and/or the level of hydration are also validated.
- Structural differences in the iron core have been associated with variations in the anions present (phosphate or sulfate), and with the electrochemical properties of iron.
- Anion concentrations in turn could reflect both the solvent composition and the properties of the protein coat.
- Formation of ferritin core is a representative example of biomineralization process.

Mechanism of Fe-core formation

- Fe(II) and dioxygen are required for core formation especially in the early stages.
- The core is formed from aqueous Fe(II) & its oxidation to Fe(III) follows its incorporation.
- Fe gets into the core through channels in the protein (proposed) and then transferred into the cavity to form first diiron-oxo dimers.
- Aggregates and clusters are formed via a progression of oligomers related to iron hydrolysates.
- Oxidation to Fe(III) and hydrolysis produce one electron and 2.5 protons for incorporation of each Fe-atom into the iron core (for 4500 Fe-atoms, 4500 electrons & 11250 protons)
- The protons are released & electrons are transferred to dioxygen (the relative rates of proton release, oxo-bridge formation, & electron transfer are not known in detail).
- If all the protons were retained, the pH would drop to 0.4

- When large numbers of Fe(II) atoms are added, the protein coat appears to stabilize the encapsulated Fe(II).
- An Fe(III)-tyrosinate (Fe(III)-ligand) complex is proposed to be a transient precursor to polynuclear cluster formation.



Iron storage mechanism (proposed)

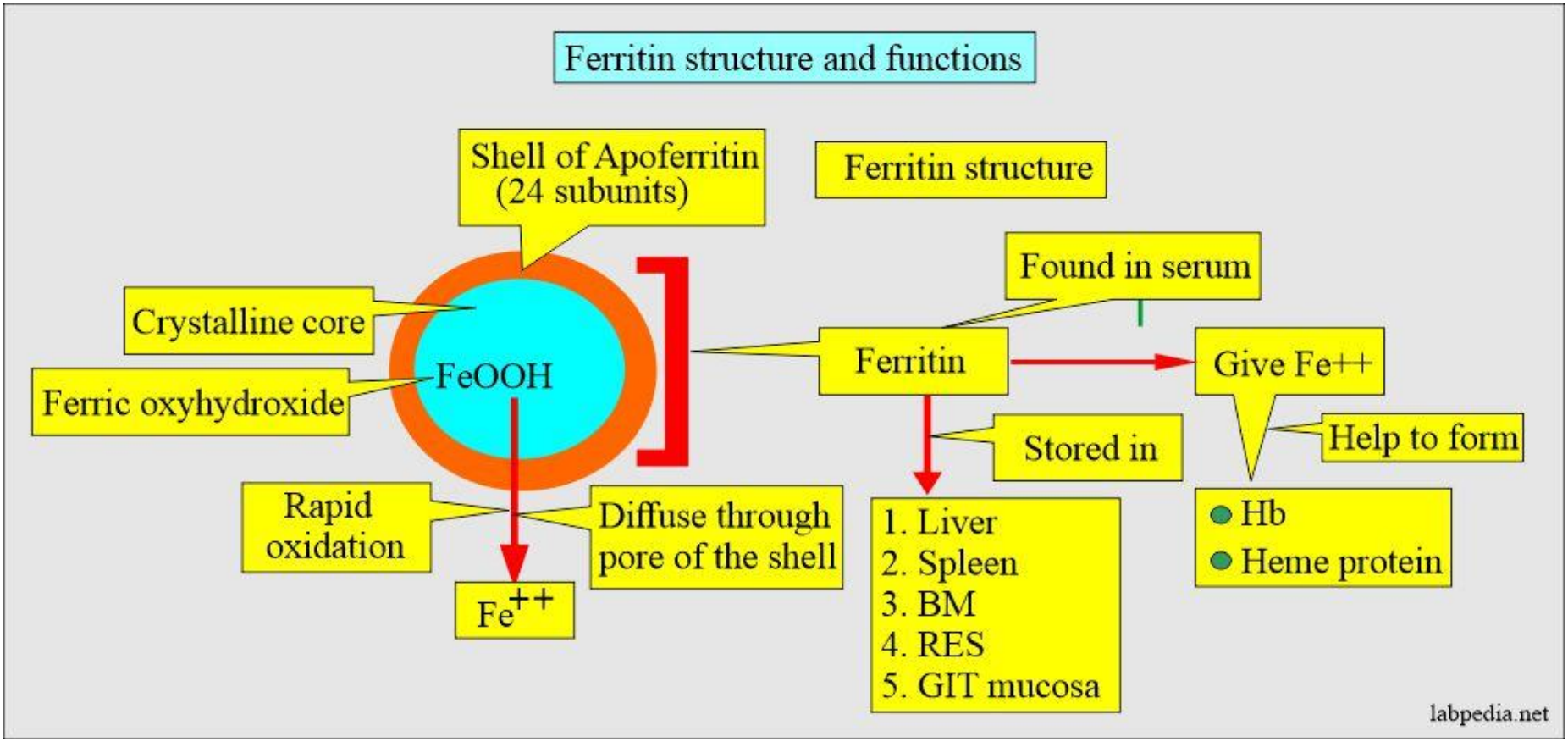
- The mechanism for the reversible incorporation of Fe in ferritin involves its transport in and out as Fe(II).
- There are 8 hydrophilic and 6 hydrophobic pores of apoferritin.
- The 24 subunits arranged in such a way that at 8 places, 3 subunits meet with their N-ends to form a polar channel (hydrophilic pores) through which Fe can be transferred in or out.
- There are also 6 non polar channels (hydrophobic pores) produced by the meeting of four subunits with their E-ends.
- Iron is taken up in the labile ferrous form prior to oxidation.
- Release of iron requires reduction of the core (Fe(III) to Fe(II)) with a biological reductant (NADH – nicotinamide adenine dinucleotide + hydrogen)

- The mobile Fe(II) is oxidized to Fe(III) at specific di-iron binding sites known as 'ferroxidase centers', present in each of the subunits.

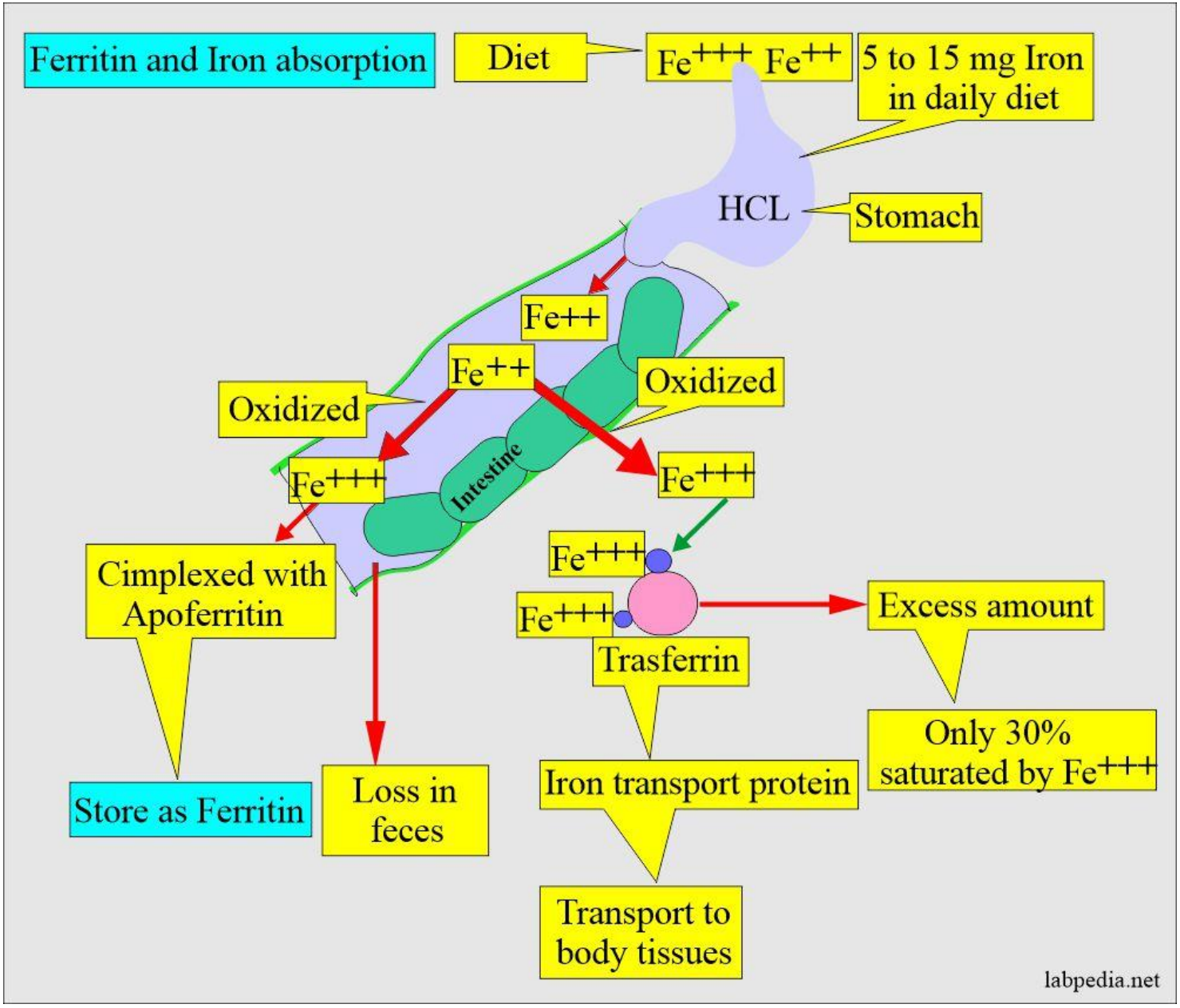
- Oxidation to Fe(III) involves the coordination of O_2 and inner sphere electron transfer:

$$2Fe(II) + O_2 + H^+ \rightarrow 2Fe(III) + H_2O_2$$

Summary of Ferritin structure and functions



Summary of ferritin and Iron absorption



Iron transport -Transferrin (Tf)

- Transport of Fe-from ferritin or hemosiderin occurs via the serum-transport protein transferrin.
- Serum transferrin is a monomeric glycoprotein with high MW ≈ 80000 D
- Serum transferrin (blood plasma - most studied), conalbumin or ovatransferrin (egg white) and lactoferrin (mother's milk) are examples of Fe-transport protein.
- Tf is also present in tears, serving to cleanse eyes after irritation.
- Transferrin is bilobal, with each lobe reversibly (essentially independently) binding Fe(III) ion (binding constant $>10^{20}$ M^{-1})
- This complexation of the metal cation occurs via prior complexation of a synergistic HCO_3^- or CO_3^{2-} ion.
- LMCT from phenolate to Fe accounts for salmon pink color of transferrin
- Binding is pH dependent, affinity for Fe(III) decreases progressively with decreasing pH.

- Ovatransferrin acts as antibacterial agent, lactoferrin is a potent antibacterial Tf (protects from infectious disease) and serum Tf is a potential Fe-transporter.
- Tf binds only two Fe-atoms & relatively efficient, as it is used in many cycles of iron transport in its interaction with the tissues to which it delivers iron.
- The crystal structure of lactoferrin and the structure of a mammalian transferrin have also reported.

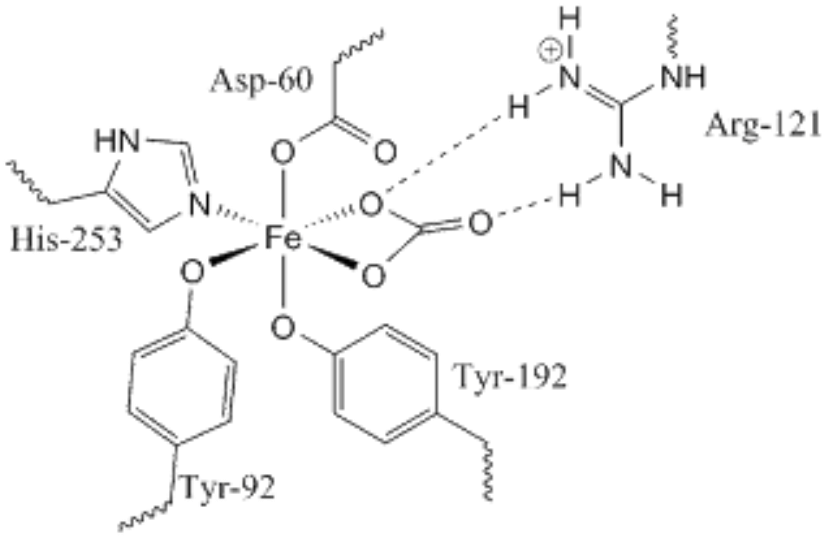
Apo-transferrin

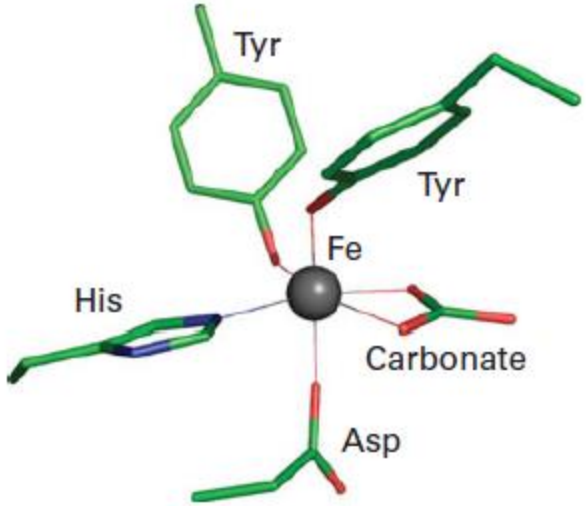
- Transferrin not bound to iron (free Tf)
- Has two iron binding sites per molecule
- For each site, the binding constant (K_A) under physiological condition is 10^{26}
- Although the two iron-binding sites of transferrin are sufficiently different, their coordination environments are quite similar.

Structure of Transferrin

- Fe site in human lactoferrin has been determined by XRD (E. Baker *et al.*)
- Transferrin is an ellipsoidal protein with two subdomains or lobes (almost similar), each of which binds iron. Polypeptide chain contains 679 amino acids.
- Serum transferrin contains about 6% carbohydrates linked to the protein, and affect the recognition & conformation of the native protein.
- Fe-atom in Tf is coordinated to four amino acid residues viz. two tyrosines (one phenolate oxygen each), one histidine (N-atom) & one aspartate (O-atom of carboxylate group).
- A bidentate CO_3^{2-} ion occupies the remaining two sites of the distorted octahedral environment.
- It sits in a pocket between the Fe-atom & two positively charged protein chains viz. an arginine side chain and a helix N-terminal.

- Sometime, a carboxylate ligand can also bind instead of CO_3^{2-} ion.
- Fe is buried deep in cleft between two protein domains, with two polypeptide strands behind it.
- Flexing of these strands alters the conformation providing driving force for Fe-release





Binding and transport/release of Fe

- Fe(II) in stomach is oxidized to Fe(III), catalyzed by a Cu-protein, ceruloplasmin
- On passing from stomach to blood (pH=7.4), oxidation of Fe(II) occurs
- Fe(III) binds to apo transferrin effectively (no other bioligand can compete)
- Fe(III) complexation involves binding of CO_3^{2-} and release of proton.

$$\text{apo-Tf} + \text{Fe(III)} + CO_3^{2-} \rightarrow \text{Fe(III)-Tf-}CO_3^{2-} + H^+$$

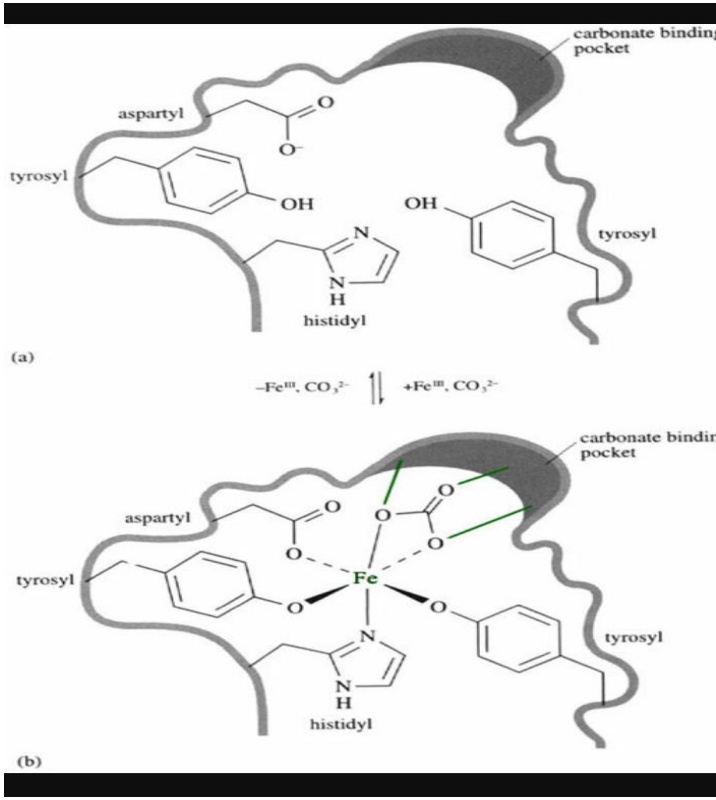
- The protein chain provides four binding sites with two cis-sites vacant for the synergistic CO_3^{2-} ion (bidentate coordination, may also be linked to Fe(III) release)
- The release of the Fe from Tf occurs at low pH of the endosome (inside the cell), and Apo-Tf is returned to the outside of the cell for delivering of another pair of Fe-atoms (millions of Fe-atoms per cell per minute)
- The reduction potential of Tf (-0.05V) is too negative to be reduced by common biological reducing agents

Release of iron from transferrin

- Cells in need of Fe produce **transferrin receptor** (plasma membrane) that binds Fe-loaded transferrin.
- The Fe-loaded transferrin receptor complex entering the cell by a process known as **endocytosis**.
- In endocytosis, a section of cell membrane is engulfed by the wall, along with its component membrane-bound proteins, to form a vesicle.
- The pH within this vesicle is then lowered by a membrane-bound H^+-pumping enzyme that is also swallowed by the cell.
- The release of Fe(III) is linked to the coordination of CO_3^{2-}, which is synergistic (it is necessary for the binding of Fe but is unstable at low pH)
- The vesicle then splits and the TF-receptor complex is returned to the plasma membrane by **exocytosis**, and Fe(III), probably now complexed by citrate, is released to the cytoplasm.

Synergistic action of CO_3^{2-}/HCO_3^-

- Without CO_3^{2-} within the coordination sphere of Fe(III), transferrin fails to retain Fe(III)
- CO_3^{2-} establishes the stabilizing interaction from H-bonding (folding protein chain) and coordination of Fe(III) in the pocket
- The anion bridges between Fe(III) and the cationic sites of encircling protein (Fe-O hard-hard interaction).
- This coordinated synergistic anion minimizes the electrostatic repulsion between the metal center and the cationic sites of the protein chain.
- Removal of CO_3^{2-}/HCO_3^- from Tf destabilizes Fe-Tf interaction, a key step in Fe-release
- This explains the synergistic function of the anion.



Problems for Practice

Q.1. Explain why the intravenous fluid used in hospital procedures contains NaCl?