1.5: Transport of Iron

The storage of iron in humans and other mammals has been dealt with in the previous section. Only a small fraction of the body's inventory of iron is in transit at any moment. The transport of iron from storage sites in cellular ferritin or hemosiderin occurs via the serum-transport protein transferrin. The transferrins are a class of proteins that are bilobal, with each lobe reversibly (and essentially independently) binding ferric ion.[37-39] This complexation of the metal cation occurs via prior complexation of a synergistic anion that *in vivo* is bicarbonate (or carbonate). Serum transferrin is a monomeric glycoprotein of molecular weight 80 kDa. The crystal structure of the related protein, lactoferrin,[39] has been reported, and recently the structure of a mammalian transferrin[40] has been deduced.

Ferritin is apparently a very ancient protein and is found in higher animals, plants, and even microbes; in plants and animals a common ferritin progenitor is indicated by sequence conservation.[41] In contrast, transferrin has been in existence only relatively recently, since it is only found ia the phylum Chordata. Although the two iron-binding sites of transferrin are sufficiently different to be distinguishable by kinetic and a few other studies, their coordination environments have been known for some time to be quite similar. This was first discovered by various spectroscopies, and most recently was confirmed by crystal-structure analysis, which shows that the environment involves two phenolate oxygens from tyrosine, two oxygens from the synergistic, bidentate bicarbonate anion, nitrogen from histidine, and (a surprise at the time of crystal-structure analysis) an oxygen from a carboxylate group of an aspartate.[39]

The transferrins are all glycoproteins, and human serum transferrin contains about 6 percent carbohydrate. These carbohydrate groups are linked to the protein, and apparently strongly affect the recognition and conformation of the native protein.

Although transferrins have a high molecular weight and bind only two iron atoms, transferrin is relatively efficient, because it is used in many cycles of iron transport in its interaction with the tissues to which it delivers iron. Transferrin releases iron *in vivo* by binding to the cell surface and forming a vesicle inside the cell (endosome) containing a piece of the membrane with transferrin and iron still complexed. The release of the iron from transferrin occurs in the relatively low pH of the endosome, and apoprotein is returned to the outside of the cell for delivery of another pair of iron atoms. This process in active reticulocytes (immature red blood cells active in iron uptake) can turn over roughly a million atoms of iron per cell per minute.[38] A schematic structure of the protein, deduced from crystal-structure analysis, is shown in Figure 1.11.

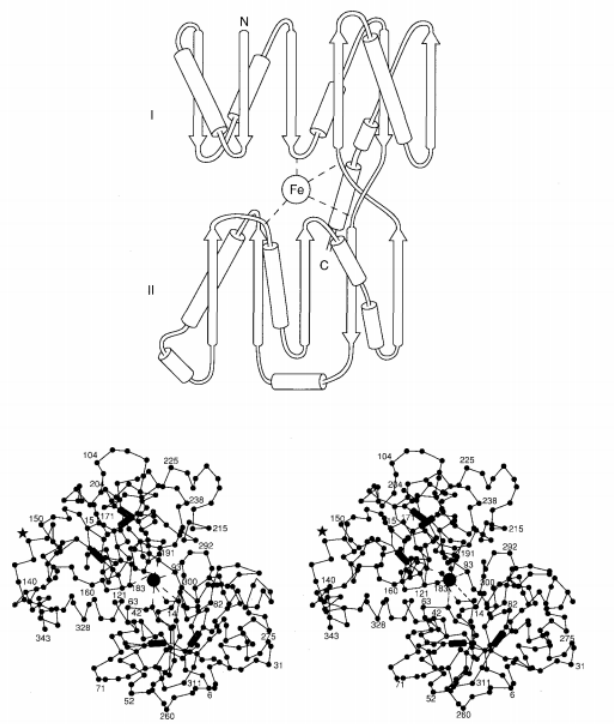

Figure 1.11: Three-dimensional structure of lactotransferrin. Top: schematic representation of the folding pattern of each lactoferrin lobe; Domain 1 is based on a beta-sheet of four parallel and two antiparallel domains; Domain II is formed from four parallel and one antiparallel strand. Bottom: stereo Ca diagram of the N lobe of lactoferrin; (●) *iron atom between domain 1 (residues 6 - 90+) and domain II (residues 91 - 251);* (⬛) *disulfide bridges;* (★) *carbohydrate attachment site. See Reference 39.*

Transferrin is an ellipsoidal protein with two subdomains or lobes, each of which binds iron. The two halves of each subunit are more or less identical, and are connected by a relatively small hinge. In human lactoferrin, the coordination site of the iron is the same as the closely related serotransferrin site. A major question that remains about the mechanism of iron binding and release is how the protein structure changes in the intracellular compartment of low pH to release the iron when it forms a specific complex with cell receptors (transferrin binding proteins) and whether the receptor protein is active or passive in the process. Recent studies suggest that the cell binding site for transferrin (a membrane, glycoprotein called the transferrin receptor) itself influences the stability of the iron-transferrin complex. The path of iron from the endosome to Feproteins has not been established; and the form of transported intracellular iron is not known.

Another major type of biological iron transport occurs at the biological opposite of the higher organisms. Although almost all microorganisms have iron as an essential element, bacteria, fungi, and other microorganisms (unlike humans and other higher organisms) cannot afford to make high-molecular-weight protein-complexing agents for this essential element when those complexing agents would be operating extracellularly and hence most of the time would be lost to the organism. As described earlier, the first life forms on the surface of the Earth grew in a reducing atmosphere, in which the iron was substantially more available because it was present as ferrous-containing compounds. In contrast to the profoundly insoluble ferric hydroxide, ferrous hydroxide is relatively soluble at near neutral pH. It has been proposed that this availability of iron in the ferrous state was one of the factors that led to its early incorporation in so many metabolic processes of the earliest chemistry of life.[6,38] In an oxidizing environment, microorganisms were forced to deal with the insolubility of ferric hydroxide and hence when facing iron deficiency secrete high-affinity iron-binding compounds called siderophores (from the Greek for iron carrier). More than 200 naturally occurring siderophores have been isolated and characterized to date.[42]

Most siderophore-mediated iron-uptake studies in microorganisms have been performed by using cells obtained under iron-deficient aerobic growth conditions. However, uptake studies in *E. coli* grown under anaerobic conditions have also established the presence of siderophore-specific mechanisms. In both cases, uptake of the siderophore-iron complex is both a receptor- and an energy-dependent process. In some studies the dependence of siderophore uptake rates on the concentration of the iron-siderophore complex has been found to conform to kinetics characteristic of protein catalysts, i.e., Michaelis-Menten kinetics. For example, saturable processes with very low apparent dissociation constants of under one micromolar (1 μM) have been observed for ferric-enterobactin transport in *E. coli* (a bacterium), as shown in Figure 1.12.

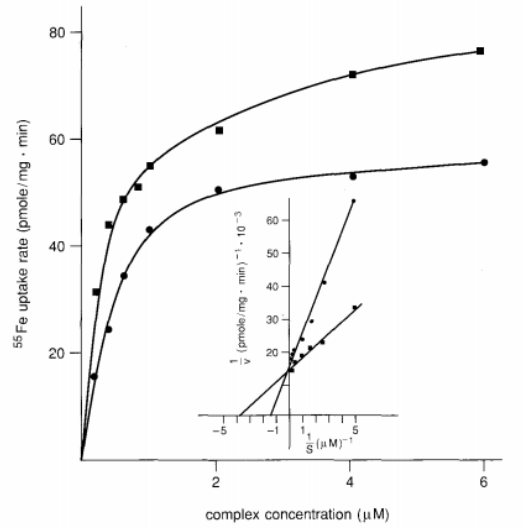

Figure 1.12 - Effect of MECAM analogues on iron uptake from E. coli. Iron transport by 2 μM ferric enterobactin is inhibited by ferric MECAM.

Similarly, in a very different microorganism, the yeast *Rhodoturala pilimanae*, Michaelis-Menten kinetics were seen again with a dissociation constant of approximately 6 μM for the ferric complex of rhodotoroulic acid; diagrams of some representative siderophores are shown in Figure 1.13. The siderophore used by the fungus *Neurospora crassa* was found to have a dissociation constant of about 5 μM and, again, saturable uptake kinetics.

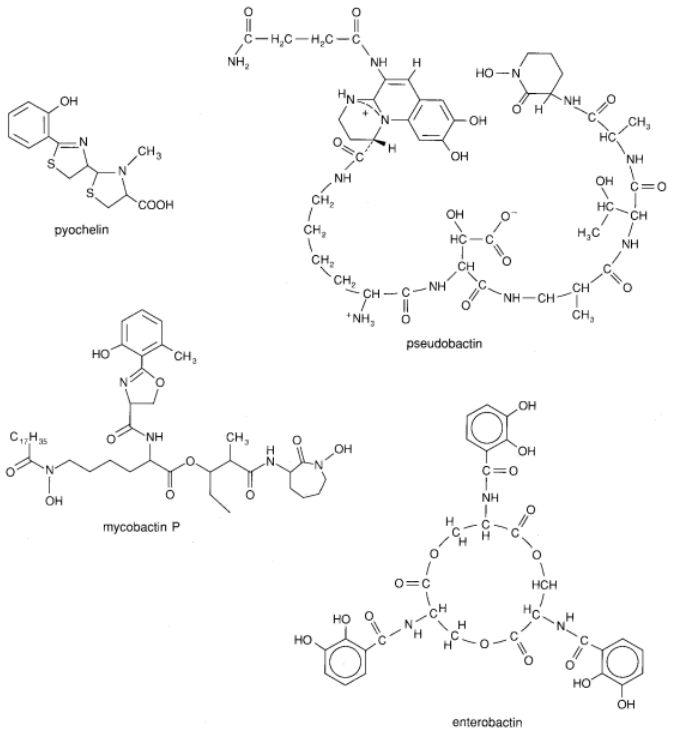

Figure 1.13 - Examples of bacterial siderophores. See Reference 42.

Although the behavior just described seems relatively simple, transport mechanisms in living cells probably have several more kinetically distinct steps than those assumed for the simple enzyme-substrate reactions underlying the Michaelis-Menten mechanism. For example, as ferric enterobactin is accumulated in *E. coli*, it has to pass through the outer membrane, the periplasm, and the cytoplasm membrane, and is probably subjected to reduction of the metal in a low-pH compartment or to ligand destruction.

A sketch of a cell of E. coli and some aspects of its transport behavior are shown in Figure 1.14. Enterobactin-mediated iron uptake in *E. coli* is one of the best-characterized of the siderophore-mediated iron-uptake processes in microorganisms, and can be studied

as a model. After this very potent iron-sequestering agent complexes iron, the ferric-enterobactin complex interacts with a specific receptor in the outer cell membrane (Figure 1.14), and the complex is taken into the cell by active transport. The ferric complexes of some synthetic analogs of enterobactin can act as growth agents in supplying iron to *E. coli*. Such a feature could be used to discover which parts of the molecule are involved in the sites of structural recognition of the ferric-enterobactin complex. Earlier results suggested that the metal-binding part of the molecule is recognized by the receptor, whereas the ligand platform (the triserine lactone ring; see Figure 1.13) is not specifically recognized.

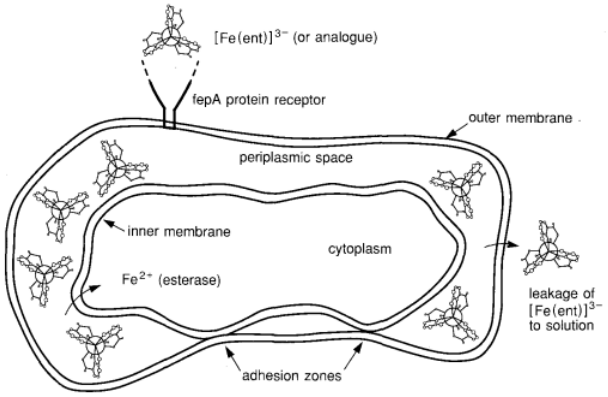

Figure 1.14 - Model for enterobactin-mediated Fe uptake in E. coli.

To find out which domains of enterobactin are required for iron uptake and recognition, rhodium complexes were prepared with various domains of enterobactin (Figure 1.15) as ligands to use as competitors for ferric enterobactin.[44] The goal was to find out if the amide groups (labeled Domain II in Figure 1.15), which linked the metal-binding catechol groups (Domain III, Figure 1.15) to the central ligand backbone (Domain I, Figure 1.15), are necessary for recognition by the receptor protein. In addition, synthetic ligands were prepared that differed from enterobactin by small changes at or near the catecholate ring. Finally, various labile trivalent metal cations, analogous to iron, were studied to see how varying the central metal ion would affect the ability of metal enterobactin complexes to inhibit competitively the uptake of ferric enterobactin by the organism. For example, if rhodium MECAM (Figure 1.16) is recognized by the receptor for ferric enterobactin on living microbial cells, a large excess of rhodium MECAM will block the uptake of radioactive iron added as ferric enterobactin. In fact, the rhodium complex completely inhibited ferric-enterobactin uptake, proving that Domain I is not required for recognition of ferric enterobactin.

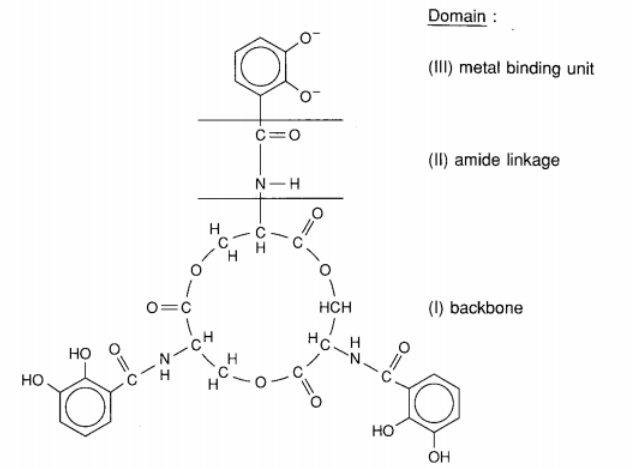

Figure 1.15 - Definition of recognition domains in enterobactin.

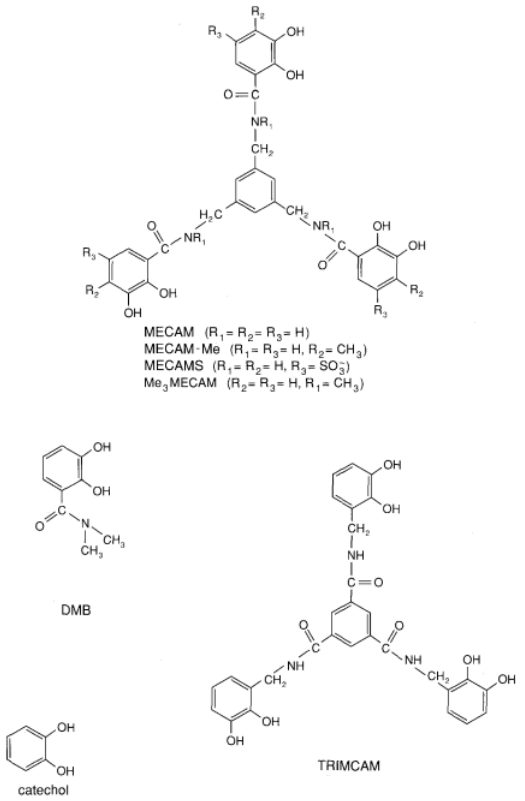

Figure 1.16 - MECAM and related enterobactin analogues.

However, if only Domain III is important in recognition, it would be expected that the simple tris(catecholato)-rhodium(III) complex would be an equally good inhibitor. In fact, even at concentrations in which the rhodium-catechol complex was in very large excess, no inhibition of iron uptake was observed, suggesting that Domain II is important in the recognition process.

The role of Domain II in the recognition process was probed by using a rhodium dimethyl amide of 2,3-dihydroxybenzene (DMB) as a catechol ligand, with one more carbonyl ligand than in the tris(catecholato)-rhodium(III) complex. Remarkably, this molecule shows substantially the same inhibition of enterobactin-mediated iron uptake in *E. coli* as does rhodium MECAM itself. Thus, in addition to the iron-catechol portion of the molecule, the carbonyl groups (Domain II) adjacent to the catechol-binding subunits of enterobactin and synthetic analogs are required for recognition by the ferric-enterobactin receptor. In contrast, when a methyl group was attached to the "top" of the rhodium MECAM complex, essentially no recognition occurred.

In summary, although the structure of the outer-membrane protein receptor of *E. coli* is not yet known, the composite of the results just described gives a sketch of what the ferric-enterobactin binding site must look like: a relatively rigid pocket for receiving the ferric-catecholate portion of the complex, and proton donor groups around this pocket positioned to hydrogen bond to the carbonyl oxygens of the ferric amide groups. The mechanisms of iron release from enterobactin, though followed phenomenologically, are still not known in detail.