Iron uptake systems of different organisms and their role in infection.

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ABSTRACT

Iron is an oxidant as well as a nutrient for almost all organisms. A proper balance of this essential nutrient is vital for good health. Its deficiency can lead to many physiological and metabolic disorders while excessive iron in specific tissues of the host (iron loading) promotes infection and other health issues. Iron can also increase disease risk by functioning as a readily available essential nutrient for invading microbial cells (which is essential for their survival and replication). The ability of pathogens to obtain iron from transferrins, ferritin ,hemoglobin and other iron containing proteins of their hosts is crucial for their survival or death. Sequestration of iron is a mechanism to control microbial proliferation and virulence. This paper reviews the iron uptake systems of a variety of organisms and discusses their role in infection.

KEYWORDS: Iron Uptake, Iron Transport, Host, Infection, Nutrition, Immunity.

INTRODUCTION

Role of iron in cellular homeostasis

Iron is an essential element for the growth of almost all the microorganisms. Nonpathogenic lactobacilli are the only organisms that do not require iron for their growth. Studies showed that iron is indispensable for DNA synthesis in organisms [Cazzola *et al.*, 1990] and iron starvation arrests proliferation of cell, mainly due to its requirement by ribonucleotide reductase enzyme and other enzymes that are involved in cell division [Hoffbrand *et al.*, 1976]. Iron is incorporated into the heme complex in higher organisms and fungi, which is an essential component of cytochrome proteins that mediate redox reactions in cell. These reactions contribute to the essential functions of cell such as mitochondrial energy metabolism, electron transport, and detoxification of toxic oxidants [Wooldridge and Williams, 1993]. Iron is an essential component of oxygen carrier proteins such as hemoglobin, myoglobin, and leghemoglobin where it acts like an indispensable cofactor for their oxygen binding capacity [(Jameson and Ibers, 1994]. Inorganic iron is also important in redox reactions in the iron-sulfur clusters of many enzymes like nitrogenase and hydrogenase. Non-heme iron proteins includes the enzyme methane monooxygenase that oxidizes methane to methanol [Rocklin *et al.*, 1999; Wallar *et al.*, 1996], ribonucleotide reductase that reduces ribose to deoxyribose which is involved in DUA biosynthesis [Hoffbrand *et al.*, 1976], hemerythrins that is involved in oxygen transport and fixation in marine invertebrates [Kurtz *et al.*, 1977] and purple acid phosphatase that is involved in hydrolysis of

phosphate esters [Stenkamp *et al.,* 1984]. Iron is a cofactor of iron superoxide dismutase in microorganisms that plays an important role in protection against the oxidative damage. [Paramchuk *et al.,* 1997]. Thus, due to its involvement in many necessary functions, iron becomes an essential nutrient for the cell.

Even though iron has a crucial role in cellular physiology, it can also serve a detrimental role to the cell by catalyzing the formation of the highly reactive hydroxyl radical or oxidants of similar reactivity from hydrogen peroxide via the Fenton reaction [Winterbourn, 1995]. The hydroxyl radical initiates a range of toxic reactions including peroxidation of biological membranes and damage to DNA [Wooldridge and Williams, 1993], though it contributes to phagocyte mediated microbicidal activity [Miller and Britigan, 1997] of cell that may be helpful in fighting against pathogens but very harmful if remains uncontrolled. Thus, iron uptake and storage in each organism should occur in such a manner that these processes may fulfill the requirement in each without causing oxidant mediated damage. Since, cellular iron content is mainly regulated at the level of uptake, iron uptake is tightly regulated in all organisms.

Iron uptake system in different organisms

In *Sacchromyces cerevisiae,* two systems of iron uptake are present. One is a low affinity iron uptake mechanism and the other is a high affinity iron uptake mechanism [Askwith *et al.,* 1996]. Both systems require ferrireductase enzymes that reduce f'erric complexes including siderophores at the first step of uptake mechanism [Askwith *et al.,* 1996; Philpott and Protchenko, 2008]. This step has a low specificity and affinity and it makes cells potent to convert structurally diverse extracellular ferric complexes into the common ferrous state [Kosman, 2003]. Yeast cell utilizes both systems according to iron concentration in environment [Askwith *et al.,* 1994]. Low affinity iron uptake system works when there is a high concentration of iron. In high concentration of iron, Fet4p, an ATP-independent transporter causes the uptake of iron [Dix *et al.,* 1994]. On the other hand high affinity iron uptake system works when there is a low concentration of iron. This system involves an oxidase—permease complex composed of the Fet3p and Ftr1 proteins in the plasma membrane [Philpott, 2006]. The Fet3p component is a multicopper oxidase that behaves like a ferroxidase and converts ferrous to ferric iron [Askwith *et al.,* 1994]. And Ftr1 is a permease that causes uptake of this iron [Stearman *et al.,* 1996]. This Oxidation-permeation pathway is a conserved process involved in iron transport across membranes in other yeasts like *Candida* and other organisms also like green algae [Herbik *et al.,* 2002; Philpott, 2006; Askwith *et al.,* 1996]. Fet3p synthesis occurs in post Golgi compartment and proper synthesis requires copper. This copper comes inside the cell and reaches to compartment via the cuprous permease, Ctr1, which also requires an activity of an intracellular cupric transporter, CCC2 [Weissman *et al.,* 2002; Yuan *et al.,* 1995]. Studies done with the mutants lacking Ctrl or CCC2 copper transport activity are found to have a defective high-affinity iron uptake system [Weissman *et al.,* 2002; Marvin ef *al.,* 2004]. Yeast can also utilize siderophores for iron uptake that do not require ferrireductase enzyme. *C. albicans* that can not produce it own siderophores expresses a specific transporter for ferrichrome-type siderophores, Aml (also known as Sit1), and use siderophores produced by other microorganisms like *S. cerevisiae* [Lesuisse and Labbe, 1989]. In *Arabidopsis,* a reductase FRO2 enzyme reduces different ferric form sources those are present in the environment [Robinson *et al.,* 1999]. Ferrous form is then internalized by a ferrous form transporter encoded by IRT1 and IRT2 [Eide *et al.,* 1996; Vert *et al.,* 2001]. Both transporters have been shown to be involved in

the iron transport. Due to its expression and localization in external cell layers of the root sub apical zone, IRT2 was suggested to be involved in the iron uptake into the roots [Vert *et al.,* 2001]. In addition, the existence of six genes encoding NRAMP-like proteins was reported in *Arabidopsis.* It was shown that AtNRAMP1 [Curre *et al.,* 2000] and AtNRAMP3 and AtNRAMP4 [Thomine *et al.,* 2000] complemented the yeast fet3/fet4 mutant and that the AtNRAMP1 accumulated in response to iron deficiency, whereas, AtNRAMP3 and AtNRAMP4 were induced by iron starvation. Other possible iron transporters in *Arabidopsis* are encoded by eight genes homologous to YS1 (yellow stripe), first described in maize *(Zea mays)* and shown to catalyze the iron uptake from ferric form phytosiderophore complexes [Curre *et al.,* 2001]. In *Chlamydomonas reinhardtii,* induction of a ferric reductase and iron uptake activity by iron deficiency has been characterized. Furthermore, a multicopper oxidase has been found that is required for high affinity iron uptake [Herbik *et al.,* 2002]. This mechanism of high affinity iron uptake in C. *reinhardtii* resembled the mechanism found in yeast and not that found in higher plants [Herbik *et al.,* 2002]. In humans and other vertebrates, dietary iron is most often present as ferric form, which is reduced to the more soluble ferrous form by a heme containing ferrireductase (DcytB), which transports electrons from cytosolic NADPH to extracellular acceptors such as ferric form of iron [McKie *et al.,* 2001]. A transmembrane permease, Nramp2 (DMT1 or DCT I) transports ferrous form of iron across the intestinal surface [Fleming *et al.,* 1997; Gunshin, *et al.,* 1997]. The iron that is not store in ferritin, is transported across the basolateral surface by a transmembrane permease termed ferroportin (IREGI, MTP, and S1c11a3) [Abboud and Haile, 2000; Donovan *et al.,* 2000; McKie *et al.,* 2001]. This process is regulated by hepcidin, which binds to ferroportin and induces its internalization [Dunn *et al.,* 2007]. Studies on gene deletion of hepcidin resulted in massive iron over loading [Nicolas *et al.,* 2001] in experimental animals. Transgenic expression of hepcidin in animals led to severe anemia [Nicolas *et al.,* 2002]. For effective utilization of iron, ferrous form that is not bound to ferritin is exported from the intestine must be converted to ferric form and subsequently bound to transferrin. Transferrin is a glycoprotein, which can bind two atom of ferric form with a high affinity at physiological conditions and this activity confirm that there is no free iron remains in blood [Wessling and Resnick, 2000]. This protein found in plasma, vitreous fluid, and brain. At this step conversion of ferrous form to ferric form is catalyzed by a multicopper oxidase hephaestin, [Vulpe *et al.,* 1999] which is present at the basolateral surface of the intestine. Mutations studies in hephaestin in mice showed that disruption of the gene leads to defective intestinal iron transport resulting in microcytic anemia [Chen *et al.,* 2006]. Most mammalian cells rely on the plasma multicopper oxidase Ceruloplasmin for oxidation of newly exported ferrous form to ferric form [Hellman and Gitlin, 2002]. Ceruloplasmin is the major copper containing protein in plasma that is involved in iron recirculation. Decreased plasma ceruloplasmin results in malfunctioning of iron homeostasis and increased tissue iron content. For example, one of the phenotypic manifestations of ceruloplasmin mutations in humans is ataxia due to iron accumulation in basal ganglia [Miyajima *et al.,* 2001]. Ceruloplasmin also helps iron to load apo transferrin to make it holo transferrin [Osaki *et al.,* 1966]. This holo-transferrin then binds to the transferrin receptor on cell surface and endocytoized by the cell. In endosomes acidification occurs during maturation and this endosomal acidification seems to cause releases of ferric form atoms from transferrin. This ferric form is then believed to convert into ferrous form by endosomal reductase enzymes [Ohgami *et al.,* 2005]. Ferrous form is more soluble in biological fluids than ferric form, but it is very toxic because it can generate highly

reactive hydroxyl radicals in the presence of oxygen, by the Fenton reaction, which can damage cell [Winterbourn, 1995]. Thus, the uptake, storage, and efflux of iron are tightly regulated by means of different sensing mechanisms in cell that can sense the concentration of iron in cell and accordingly regulates its homeostasis [Wessling and Resnick, 2000]. Iron metabolism is regulated mainly by two iron regulatory proteins [Hentze *et al.*, 1996]. These proteins bind to iron-responsive elements (IREs) of mRNAs that encode proteins involved in iron uptake (TfR1 and DMT1), utilization (erythroid d-aminolevulinic acid synthase), storage (ferritin) and export (ferroportin-1). Storage of iron in ferric form in ferritin helps in the avoiding the toxicity of iron in cell. For this storage of iron in ferritin, it is translocated from endosome to cytosol by the divalent cation efflux pump Nramp2 (S1c11a2/DMT1) [Gruenheid *et al.*, 1999**;** Picard *et al.*, 2000]. Nramp2 knockouts cause postnatal death of experimental organisms due to a severe deficiency in intestinal iron absorption [Gunshin *et al.*, 2005]. This experiment shows the central role of Nramp2 in providing iron for essential metabolic pathways in the cytosol. With Nramp 2, macrophages also express an additional metal transporter, Nramp I (S1c11a1), in their late endosomes/lysosomes. Nrampl was initially thought for iron influx into phagolysosomes [Goswami *et al.*, 2001] for killing the pathogen. This was thought to do so because of its discovery by increased susceptibility of its knockout for intracellular parasites such as *Salmonella*, *Mycobacteria* and *Leishmania* [Blackwell, 2001; Forbes and Gros, 2001]. But now it is believed that it also functions as a pH dependent divalent cation efflux pump, similar to Nramp2 [Jabado *et al.*, 2000]. Now the increased susceptibility to infection of Nrampl knock-outs is argued as by restricting the access of iron to pathogen [Weinberg, 1992; Jabado *et al.*, 2000; Schaible and Kaufmann, 2004]. With it other necessary functions in human, iron is the main controller of microbicidal responses of host cell against the invading pathogens particularly in macrophage. Iron acts as double-edged sword in killing pathogens [Weiss, 2002]. On one hand, iron is involved in the production of toxic oxygen and nitrogen intermediates that kill pathogens by the Fenton reaction and on the other hand iron can increase the expression of inducible nitric oxide synthase by LPS/IFNy-activated macrophage which produce more nitric oxide that causes the down regulation of the transferrin receptor and up regulation of the ferritin. Both these events together cause the scarcity of free iron in cell, thus favoring the pathogen survival inside the host [Weiss *etal.*, 1994; Oexle *et al.*, 2003]. Production of nitric oxide is increased during infection so that host cell can cut the supply of iron for the survival of the endocytosed pathogen [Kim and Ponka, 2000].

Multicopper oxidases: central role in iron homeostasis

Multicopper oxidases (MCOs) are very essential component of high affinity iron uptake system for many organisms. This is a diverse family of metalloenzymes widely distributed among eukaryotes, which is characterized by distinctive structural, spectroscopic, and enzymatic properties [Solomon *et al.*, 1996]. In MCO, specialized copper sites have been recruited during evolution to provide long-range electron transfer reactivity and oxygen binding and activation in proteins destined to cope with oxygen reactivity in different organisms. As described already that uptake of iron in *S. cerevisiae* is accomplished by an oxidase-permease complex composed of a MCO known as Fet3p which contains ferrous to ferric conversion activity or ferroxidase activity and an iron transporter known as FTR1 [Stearman *et al.*, 1996]. A similar mechanism of iron uptake requiring a MCO and an iron transporter was also identified in other yeasts like *S. pombe*

[Askwith and Kaplan, 1997] and *C. albicans* [Knight *et al.*, 2002]. In mammalian cells the mostly discussed and studied iron uptake system is Transferrin (Tf)-Transferrin receptor (TfR) pathway [Rouault and Klausner, 1997]. Here also the involvement of mammalian multicopper ferroxidase ceruloplasmin [Mukhopadhyay *et al.*, 1998, Attieh *et al.*, 1999, Qian *et al.*, 2001, Rouault and Klausner, 1997] was demonstrated for cellular iron uptake. Ceruloplasmin and its membrane bound homologue hephaestin plays a critical role in iron homeostasis as evidenced from mutations resulting into functional inefficiency of any of these ferroxidases which lead to iron related disorders. These studies confirm the importance of ferroxidases in iron biology of mammals. Furthermore, the significance of MCO has been studied in other organisms. CueO, a MCO of *E. coli* is required for copper tolerance by Cu (I) oxidase activity of the enzyme [Kim *et, al.*, 2001]. In *S. cerevisiae*, it has been observed that deletions in Fet3p gene that is involved in high affinity iron uptake system, cause iron deficiency in organism and make it unable to grow in iron-restricted condition. Moreover, deletions in the genes that facilitate high-affinity copper uptake or delivery of copper ions to the multicopper oxidase Fet3p result in strains that also display defective high-affinity iron uptake [Knight *et al.*, 1996; Lin *et al.*, 1997; Yuan *et al.*, 1995]. High affinity iron uptake has been shown to be important for virulence in C. *albicans* and a homozygous Caftrl- null mutant is unable to cause a systemic infection in a mouse model [Ramanan *et al* 2000]. The MCO mediated iron uptake into green algae *Chlamydomonas reinhardtii* [Herbik *et al.*, 2002] underscores the importance of this pathway in evolution.

Iron and infection

Pathogens require iron for their survival and proper growth inside the host. They acquire this iron from their hosts and they have to compete with host to acquire more iron for their essential enzymatic functions. Uptake of iron for these essential enzymes for survival of pathogen poses a problem for both host and parasite [Wilson and Britigan, 1998]. Human pathogens can acquire iron by two different ways. One is by coming direct contact with the host iron source and utilizes the iron by releasing it from the host protein by enzymatic method [Philpott, 2006; Wandersman and Delepelaire, 2004]. The second way is the use of siderophores that are able to remove iron from host protein-iron complexes because of their high-affinity for the iron, which is comparable of host iron binding protein [Philpott, 2006; Wandersman and Delepelaire, 2004]. Iron is required for the virulence of pathogenic yeasts like *C albicans*. *C albicans* establishes systemic infection by getting iron from heme [Weissman *et al.*, 2002; Santos *et al.*, 2003] of host by producing hemolytic factors [Manns *et al.*, 1994] that causes lysis of RBC. C *albicans* can also utilize transferrin bound iron via iron reduction pathway by ferrireductase [Knight *et al.*, 2005] in systemic infection. *C albicans* have at least three high-affinity iron uptake systems and at least two of them seem to be essential for successful development of infection in the host [Ramanan *et al.*, 2000; Heymann *et al.*, 2002]. Further studies on deletion of the high affinity iron uptake system component i.e. a permease gene (FTRI) that express as iron transporter cause elimination of the capacity of the pathogen to establish systemic infection in mice [Ramnan *et al.*, 2000]. Although, *C albicans* cannot synthesize siderophores, they have siderophore transporter called Arn1. Their uptake via Aml transporter seems to be required for colonizing epithelial layers, but is not essential for the systemic infection [Heymann *et al.*, 2002]. Many bacteria secrete siderophores that have a high affinity for iron. Theycompete with transferrin and other host proteins for ferric form [Crosa and Walsh, 2002]. Studies

showed that both the ferrous form and ferric form are required by *Salmonella enterica Typhimurium* for its virulence and survival inside the host. It is a gram- negative facultative intracellular bacterium that secretes siderophores that bind to ferric form with high affinity. These siderophores are internalized after binding to outer membrane receptors [Hantke *et al.,* 2003; Rabsch *et al.,* 2003]. Ferrous form uptake is done by ATP dependent or proton coupled transmembrane transporters on the cell. Mutation studies on these transporters showed their essentiality in proper growth and virulence of bacteria [Marquis and Gros, 1997; Janakiraman and Slauch, 2000; Boyer *et al.,* 2002]. Similar studies have been done and confirmed on *Mycobacteria.* [Agranoff *et al.,* 1999; Boechat *et al.,* 2002]. In protozoan *Tritrichomonas foetus*, the causative agent of bovine trichomoniasis, a sexually transmitted disease that's leads to abortion and eventually to permanent infertility [Robert, 2004], has high requirements for extracellular iron (50-100 mM) [Tachezy *et al.,* 1996]. Studies on the involvement of iron in the virulence were done by infection in mouse by the moderately virulent KV-1 strain with a 5% mortality rate [Kulda *et al.,* 1999]. Administration of ferric ammonium citrate to infected mice increased the mortality rate up to a level determined for the highly virulent LUB- lMIP strain with 80% mortality rate [Kulda *et al.,* 1999]. This study also showed that the strain with lower virulence has a lower efficiency in uptaking iron [Kulda *et al.,* 1999]. In trypanosomatid protozoa, receptor-mediated uptake of ferric form chelated to transferrin has been demonstrated in the African trypanosome, *Trypanosoma hrucei* [Steverding *et al.,* 1995]. The uptake occurs via the flagellar pocket and it is mediated by a receptor (hetero-dimeric glycosylphosphatidylinositol anchored receptor) consisting of subunits encoded by expression site associated genes ESAG-6 and ESAG-7 [Steverding *et al.,* 1995]. *Leishmania amazonensis,* a ZIP family transporter was described for iron uptake [Huynh *et al.,* 2006].

CONCLUSION

The survival of any organism is dependent on its ability to use iron from its immediate environment for a wide array of metabolic functions. In an aerobic environment at neutral pH iron exists in its oxidized ferric form which is poorly soluble and unavailable for uptake into cells. Ferrous form of iron can be taken up by cells, but it might be highly deleterious because of its capacity to form and react with reactive oxygen species and cause irreversible damage to bio-molecules [Gutteridge and Halliwell, 1990]. Thus, nature evolved safe strategies for iron uptake, transport across the membrane and utilization into the cells in a tightly regulated manner. The mechanism of iron uptake from bacteria to higher mammals is grossly distributed into variations of two mechanisms, either by a siderophore/transferrin specific receptor pathway and/or by an iron oxidase/permease complex. Whereas, iron uptake by siderophores or by transferrin has been known for a longer period of time, the uptake of iron mediated by a multi copper oxidase (MeO) has been reported comparatively recently, first in yeasts [Askwith et ai., 1994; Stearman et ai., 1996] and later in mammalian cells [Mukhopadhyay et aI., 1998; Attieh et ai., 1999] as well as in algae *Chiamydomonas reinhardtii* [Herbik et ai., 2002], which underscores the importance of this pathway in evolution. Iron is an essential nutrient for virtually all human pathogens, an important facet of the innate immune system is to limit iron availability to

invading microbes in a process termed nutritional immunity. Successful human pathogens must therefore possess mechanisms to circumvent nutritional immunity in order to cause disease.

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