Iron Sequestration And Bacterial Pathogens

*Nikita Choudhary And **Dr. Shivarti Gautam

*Research Scholar, Department of Biotechnology, SunRise University, Alwar, Rajasthan (India)
**Professor, Department of Biotechnology, SunRise University, Alwar, Rajasthan (India)
Email: 62024nikita@gmail.com

Abstract: Sequestration of the essential nutrient iron from bacterial invaders that colonize the vertebrate host is a central feature of nutritional immunity and the "fight over transition metals" at the host-pathogen interface. The iron quota for many bacterial pathogens is large, as iron enzymes often make up a significant share of the metalloproteome. Iron enzymes play critical roles in respiration, energy metabolism, and other cellular processes by catalyzing a wide range of oxidation-reduction, electron transfer, and oxygen activation reactions. In this Concept article, we discuss recent insights into the diverse ways that bacterial pathogens acquire this essential nutrient, beyond the well-characterized tris-catecholate Fe^{III} complexes, in competition and cooperation with significant host efforts to cripple these processes. We also discuss pathogen strategies to adapt their metabolism to less-than-optimal iron concentrations, and briefly speculate on what might be an integrated adaptive response to the concurrent limitation of both iron and zinc in the infected host.
[Nikita Choudhary; Shivarti Gautam. **Iron Sequestration And Bacterial Pathogens**. *Researcher* 2024;16(12):10-14]. ISSN 1553-9865 (print); ISSN 2163-8950 (online). http://www.sciencepub.net/researcher. 03. doi:10.7537/marsrsj161224.03

Keywords: Catechols; host-pathogen interface; iron; iron uptake; metal nutrient acquisition; siderophores.

Introduction

Iron is an essential micronutrient for both microbes and humans alike. For well over half a century we have known that this element, in particular, plays a pivotal role in health and disease and, most especially, in shaping host-pathogen interactions. Intracellular iron concentrations serve as a critical signal in regulating the expression not only of high-affinity iron acquisition systems in bacteria, but also of toxins and other noted virulence factors produced by some major human pathogens. While we now are aware of many strategies that the host has devised to sequester iron from invading microbes, there are as many if not more sophisticated mechanisms by which successful pathogens overcome nutritional immunity imposed by the host. This review discusses some of the essential components of iron sequestration and scavenging mechanisms of the host, as well as representative Gram-negative and Gram-positive pathogens, and highlights recent advances in the field. Last, we address how the iron acquisition strategies of pathogenic bacteria may be exploited for the development of novel prophylactics or antimicrobials.

BIOLOGICAL IMPORTANCE OF IRON

Iron is essential to nearly all life forms on Earth, required for the proper function of enzymes involved in, for example, respiration, photosynthesis, the tricarboxylic acid cycle, nitrogen fixation, electron transport, and amino acid synthesis. The utility of iron in biological processes hinges on its chemical properties as a transition metal, engaging in single electron transfers to interconvert between the ferrous (Fe^{2+}) and ferric (Fe^{3+}) states. While this clearly makes iron advantageous, the same property provides the explanation for why excess, or "free," iron is inherently toxic. Ferrous iron–catalyzed Fenton chemistry results in the generation of the highly toxic hydroxyl radical (OH•) that can compromise cellular integrity through damage to lipids, proteins, and nucleic acids.

Aside from *Borrelia burgdorferi* and *Treponema pallidum*, iron is essential to all microbial pathogens, yet perhaps the most difficult issue facing pathogens is accessing enough iron to support growth. The concentration of iron under physiological conditions (10^{-8} to 10^{-9} M) is orders of magnitude below the $\sim 10^{-6}$ M required for bacterial growth, owing to the formation of insoluble ferric oxyhydroxide precipitates, and host sequestration mechanisms further decrease the available concentration to the range of $\sim 10^{-18}$ M. As such, iron plays a fundamental role in host-pathogen interactions, and coevolution has shaped both bacterial and host iron acquisition/sequestration mechanisms.

IRON METABOLISM IN HEALTH AND DISEASE

Host Iron Homeostasis

Perturbations to the balance of iron within the human body can impact both overall health and susceptibility to infectious disease. Sophisticated mechanisms thus exist to control the daily intake of iron that is required for metabolic processes and the synthesis of new erythrocytes. The majority of iron within the body is found intracellularly, in association with heme, a planar tetrapyrrole ring that coordinates a central ferrous iron ion, and is bound within proteins such as hemoglobin or myoglobin. Hemoglobin is typically contained within circulating erythrocytes, but during routine destruction of senescent cells, it is released into the blood. Although the levels of extracellular hemoglobin are usually low, rapid erythrocyte lysis triggered by pathological conditions can increase the concentration of free heme and hemoglobin to harmful levels. Free hemoglobin is thus scavenged by haptoglobin, whereas hemopexin is involved in sequestration of free heme. Through receptor-mediated endocytosis, hemoglobin-haptoglobin and heme-hemopexin complexes are taken up by macrophages or hepatocytes, and the bound iron can either be stored or returned to the iron cycle.

Dietary iron is taken up by enterocytes as either heme-iron or as ferrous iron through apical surface localized heme carrier protein (HCP)-1 or divalent metal transporter (DMT)-1, respectively. Ferric iron is first reduced to ferrous iron prior to uptake, and heme is degraded by heme oxygenase to release ferrous iron. Ferrous iron faces three potential fates inside the enterocyte: (i) utilization in cellular processes, (ii) sequestration in the multimeric, iron-storage protein, ferritin, or (iii) export from the cell into the circulation via ferroportin. Ferroportin is the only known iron exporter in humans, and it functions to mobilize iron primarily from enterocytes and macrophages for transport to sites of demand within the body. Ferrous iron effluxed by ferroportin is oxidized by hephaestin on the surface of enterocytes, and ceruloplasmin on nonintestinal cells and within the plasma, thus permitting iron-loading of transferrin, which has poor affinity for Fe^{2+}.

Iron-Withholding as a Facet of Innate Immunity

Transferrin is an abundant serum glycoprotein capable of reversibly coordinating two molecules of Fe^{3+} with very high affinity ($K_d = 10^{23}\ M^{-1}$ at neutral pH). Transferrin is invaluable to iron homeostasis in humans, because it delivers iron to various cells but also scavenges free iron in the bloodstream, essentially sequestering it from invading pathogens. The transferrin-like glycoprotein lactoferrin is commonly found extracellularly in secretions and intracellularly in the secondary granules of neutrophils. Lactoferrin functions to sequester iron at mucosal surfaces and is released from neutrophils at infectious foci, thus participating in the host defense against invading microbes.

Many dynamic processes exist within the host to restrict the iron available to pathogens. The withholding of iron, and other essential metals, is referred to as “nutritional immunity” and functions as a key component of innate immunity. Much of this response is orchestrated by the human hormone hepcidin, which is secreted by the liver and directly regulates the internalization and degradation of ferroportin. Hepcidin is released primarily in response to excess levels of extracellular iron, either to combat transferrin saturation or as part of the inflammatory response. With the degradation of ferroportin, iron is stored intracellularly, dietary iron uptake is halted, and iron release from macrophages is stopped. Overall, this process may result in a decrease in serum levels by as much as 30%, and when triggered by invading microbes, is referred to as the “hypoferremia of infection.”

To combat infection by intracellular pathogens, the natural resistance macrophage protein 1 functions to export iron and other essential metals from the phagosomal compartment. Natural resistance macrophage protein 1 is also involved in the release of neutrophil gelatinase-associated lipocalin (NGAL; also known as siderocalin, lipocalin 2, and 24p3), an acute-phase protein secreted primarily by neutrophils that scavenges catechol-type siderophores produced by bacteria. NGAL binds enterobactin, a siderophore widely synthesized by the *Enterobacteriaceae*, and NGAL-deficient ($NGAL^{-/-}$) mice are hypersusceptible to Gram-negative bacterial infections (1). Despite lacking an intrinsic ability to bind iron, NGAL appears to function primarily in the maintenance of extracellular and intracellular iron concentrations within the host. The transport of iron by NGAL is purportedly aided by the mammalian siderophore, 2,5-dihydroxybenzoic acid (2,5-DHBA), which is structurally similar to the 2,3-DHBA iron-binding moiety of enterobactin (2, 3). 2,5-DHBA likely scavenges free iron and, when bound to NGAL, may be effectively shuttled across cellular membranes (2). Notably, 2,5-DHBA is capable of promoting growth of *Escherichia coli* in a mammalian model, so it is not surprising that synthesis of 2,5-DHBA is downregulated during infection, freeing NGAL to bind to bacterial siderophores.

Hereditary Diseases Compromising Iron-Withholding Within the Host

Although iron homeostasis is stringently controlled, many hereditary disease states, as well as excess exposure to the element, can result in an imbalance in iron levels, impacting both the overall health of the host and susceptibility to infectious disease. The most common genetic disease influencing host iron metabolism is hereditary hemochromatosis, which is largely caused by defects to the gene encoding human hemochromatosis protein, *HFE* (for high Fe). Although the exact molecular mechanisms governing hereditary hemochromatosis are still unclear, recent insights suggest that HFE is involved in iron-sensing and the regulation of hepcidin production (4). HFE is thought to participate in the formation of an iron-sensing complex, which in the presence of high levels of holo-transferrin activates a signal cascade promoting transcription of the gene encoding hepcidin (*HAMP*) (5). Deactivation of this regulatory complex and/or cascade results in inadequate production of hepcidin, leading to excessive intestinal iron absorption and recycling (6). The net result of the aforementioned dysregulation is abnormally high levels of free iron within the body, broadly referred to as iron overload.

Other less common hereditary iron overload diseases are principally associated with genetic defects in the genes encoding ceruloplasmin (*CP*) and transferrin (*TF*), which result in aceruloplasminemia and atransferrinemia/hypotransferrinemia, respectively (7). The absence of ceruloplasmin impairs the ability of ferroportin to export iron in cells lacking an alternate ferroxidase (e.g., hephaestin), as is the case with astrocytes of the central nervous system, which leads to iron accumulation in the brain and progressive neurodegeneration (8). Reduced or absent serum transferrin similarly impairs the trafficking of iron throughout the body, as well as the production of hepcidin (as discussed above), leading to accretion primarily within the liver and heart. Further, inadequate iron delivery to hematopoietic cells in the bone marrow by transferrin results in decreased hemoglobin availability, leading to anemia (9). The symptoms of hereditary hemochromatosis and aceruloplasminemia are most effectively delayed or reduced through phlebotomy and/or chelation therapy (5, 10), while atransferrinemia/hypotransferrinemia is managed primarily through transfusion of whole blood or purified apo-transferrin (11). Not only do diseases impacting host iron homeostasis enhance the risk of severe and invasive infections, but often so do their clinical interventions. Indeed, while thalassemia and sickle cell disease (SCD) can result in iron overload, it is often the treatment for these conditions that leads to hyperferremia. Both thalassemia and SCD are characterized by the abnormal formation of adult hemoglobin, a heterotetramer comprised of two α-globin subunits and two β-globin subunits. In thalassemia, genetic defects can impact the synthesis of either of the globin subunits (α-thalassemia and β-thalassemia), resulting in ineffective erythropoiesis and accelerated hemolysis of existing erythrocytes (12). SCD is due specifically to a single amino acid change in the β-globin subunit, which results in the polymerization of hemoglobin tetramers into rigid fibers and gives rise to the sickle-shaped erythrocytes from which the disorder derives its name (13, 14). Sickle cells are inherently fragile, and their instability contributes to the development of hemolytic anemia. As with thalassemia, the symptoms of SCD may be treated with frequent blood transfusions, which enhances not only the risk of iron overload, but also the frequency and severity of bacterial and fungal infections.

To reduce iron content in patients suffering iron overload, treatment can include phlebotomy or chelation therapy. Deferoxamine mesylate, the mesylate salt derivative of the microbial siderophore desferrioxamine B, is frequently used for chelation therapy. Deferoxamine mesylate, however, can promote more severe infection by some bacteria, including *Yersinia* spp., *Klebsiella* spp., and *Staphylococcus aureus* (15–17), which are able to use the siderophore as a source of iron.

OVERVIEW OF BACTERIAL IRON ACQUISITION STRATEGIES

Even within the healthy host, bacterial invaders that scavenge enough iron have the potential to cause infection. Iron has ultimately driven an evolutionary arms race between microbes in pursuit of this essential element and hosts striving to withhold it from them. In effect, for every mechanism employed by the host to sequester iron, bacterial pathogens have evolved mechanisms to circumvent this withholding strategy (see Fig. 1). Accordingly, a diverse array of iron acquisition strategies exist among bacterial pathogens, some of which are highly conserved and broadly employed, while others are highly specific and intricately linked to the pathophysiology of a given bacterium. Broadly, and as summarized in Fig. 1, the mechanisms for iron uptake by pathogenic bacteria include (i) the extraction and capture of heme-iron from host hemoproteins through the use of secreted proteins or cell surface–associated receptors, (ii) the acquisition of transferrin and lactoferrin-bound iron

through specific surface-associated binding proteins or the secretion of siderophores, and (iii) the uptake of free inorganic iron facilitated by ferric iron reductases and associated ferrous iron permeases. Later in the chapter, the mechanisms by which pathogens acquire iron within the host are discussed, with a focus on how these mechanisms are known to impact the virulence of bacterial pathogens.

Coordinated Regulation of Iron Acquisition and Virulence Gene Expression

Iron, or lack thereof, is a fundamental sensory cue in bacterial pathogens, and it triggers the coordinated regulation of genes involved in both iron acquisition and virulence. The response to iron deprivation in prokaryotes is largely controlled by two separate families of highly conserved, iron-responsive regulators that repress transcription of iron acquisition genes when the concentration of intracellular iron is high and alleviate this repression when iron is limiting. In Gram-negative bacteria, and the low–guanine and cytosine (G+C)–content Gram-positive *Firmicutes*, such as *Staphylococcus* spp., *Bacillus* spp., and *Listeria monocytogenes*, iron homeostasis is controlled by members of the ferric uptake regulator (Fur) superfamily (18). The high-G+C-content Gram-positive *Actinobacteria*, *Corynebacterium diphtheriae*, and *Mycobacterium* spp., utilize an alternative iron-responsive regulator, the diphtheria toxin repressor (DtxR; IdeR in the *Mycobacteriaceae*) to mediate both iron uptake and virulence gene expression (19). Notably, while little primary sequence similarity exists between members of these two families, Fur and DtxR-like proteins possess similar domain architecture and function in a comparable manner. In brief, both Fur and DtxR are homodimeric metalloregulators that bear an N-terminal helix-turn-helix DNA-binding domain and a C-terminal metal corepressor binding site that also functions in dimerization of the protein (18, 20). Coordination of Fe^{2+} within each protein subunit induces a conformational change rendering the iron-loaded repressor proficient for DNA binding, which occurs at a consensus sequence located within the promoter/operator region of the targeted gene (18, 21–23). Association of Fur-Fe^{2+} or DtxR-Fe^{2+} with the operator of iron-responsive genes effectively bars RNA polymerase from binding the promoter, thereby inhibiting transcription under iron-replete conditions. When intracellular iron concentrations are low, the metal is no longer readily available to interact with the repressor, the complex dissociates from DNA, and transcription proceeds.

The overwhelming majority of iron acquisition strategies employed by bacterial pathogens, and discussed herein, are wholly or at least partially regulated by members of the Fur or DtxR superfamilies. In addition to their role in iron acquisition, Fur and DtxR both play a key role in virulence and have been shown to directly or indirectly (e.g., through small Fur/DtxR-regulated RNAs) control the expression of factors contributing to pathogenicity, including the secretion of toxins, production of adhesins, formation of biofilms, and regulation of quorum sensing (24, 25). Indeed, DtxR was initially identified, as its name implies, as an iron-dependent negative regulator of diphtheria toxin production in *C. diphtheriae* (22, 26). Further, both Fur and DtxR have been demonstrated, through relevant *in vivo* models, to influence the colonization, survival, and/or proliferation of numerous bacterial pathogens within the host including, but not limited to, *S. aureus*, *Helicobacter pylori*, *Haemophilus influenzae*, *L. monocytogenes*, *Campylobacter jejuni*, *Vibrio cholerae*, and *Mycobacterium tuberculosis* (for reviews see 26, 27). The role of these metalloregulatory proteins *in vivo* no doubt is complex and multifactorial, but it appears to reflect a common strategy employed by bacterial pathogens in order to respond appropriately upon sensing their transition into the iron-limited host.

ACCESSING HEME IRON

While bacterial pathogens express a plethora of iron acquisition systems, capable of exploiting a multitude of host iron sources, heme represents a particularly auspicious target because it comprises approximately 75% of the total mammalian iron pool. Indeed, heme is a preferred iron source for pathogens such as *S. aureus* (28) and an obligate requirement for heme auxotrophs including staphylococcal small colony variants (29), *Bartonella* spp., *Bacteroides* spp., *Porphyromonas gingivalis*, and *H. influenzae* (30). Because heme exists primarily in association with hemoglobin within circulating erythrocytes, invading pathogens have evolved sophisticated mechanisms to access heme from intracellular hemoproteins. The secretion of hemolysins is a tactic commonly employed by extracellular and facultative intracellular pathogens to enhance local heme availability. Indeed, the expression of such cytolytic factors is often induced under conditions of iron starvation and during bacteremia and has long been recognized as an important multifactorial determinant of pathogenicity in many bacteria (31).

Upon its release from damaged erythrocytes, hemoglobin, or spontaneously dissociated heme, may

be captured by bacteria expressing specific secreted and/or cell surface–associated heme/hemoglobin-binding proteins. Additionally, some pathogens are capable of extracting heme from heme-iron complexes such as heme-hemopexin, hemoglobin-haptoglobin, and serum albumin, thus subverting the efforts of these host iron sequestration proteins in withholding iron from microbial invaders. In this section, archetypical heme acquisition systems will be discussed, as will be less-conserved methods employed by specific pathogens.

References

1. Beard JL. Iron biology in immune function, muscle metabolism and neuronal functioning. J Nutr. 2001;131:568S–80S.
2. Andrews NC. Forging a field: the golden age of iron biology. Blood. 2008;112:219–30.
3. Hentze MW, Muckenthaler MU, Galy B, et al. Two to tango: regulation of mammalian iron metabolism. Cell. 2010;142:24–38.
4. Biggs TE, Baker ST, Botham MS, et al. Nramp1 modulates iron homeostasis in vivo and in vitro: evidence for a role in cellular iron release involving de-acidification of intracellular vesicles. Eur J Immunol. 2001;31:2060–70.
5. Soe-Lin S, Sheftel AD, Wasyluk B, et al. Nramp1 equips macrophages for efficient iron recycling. Exp Hematol. 2008;36:929–37.
6. Soe-Lin S, Apte SS, Andriopoulos B, Jr, et al. Nramp1 promotes efficient macrophage recycling of iron following erythrophagocytosis in vivo. Proc Natl Acad Sci USA. 2009;106:5960–5.
7. Feder JN, Gnirke A, Thomas W, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet. 1996;13:399–408.
8. Goswami T, Andrews NC. Hereditary hemochromatosis protein, HFE, interaction with transferrin receptor 2 suggests a molecular mechanism for mammalin iron sensing. J Biol Chem. 2006;281:28494–8.
9. Schmidt PJ, Toran PT, Giannetti AM, et al. The transferrin receptor modulates Hfe-dependent regulation of hepcidin expression. Cell Metab. 2008;7:205–14.
10. Gao J, Chen J, Kramer M, et al. Interaction of the hereditary hemochromatosis protein, HFE, with transferrin receptor 2 is required for transferrin-induced hepcidin expression. Cell Metab. 2009;9:217–27.
11. Andriopoulos B, Corradini E, Xia Y, et al. BMP6 is a key endogenous regulator of hepcidin expression and iron metabolism. Nat Genet. 2009;41:482–7.
12. Meynard D, Kautz L, Darnaud V, et al. Lack of BMP6 induces massive iron overload. Nat Genet. 2009;41:478–81.
13. Ganz T, Nemeth E. Hepcidin and disorders of iron metabolism. Annu Rev Med. 2011;62:13, 1–4.
14. Nemeth E, Tuttle MS, Powelson J, et al. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science. 2004;306:2090–3.

10/25/2024