



Review

**Iron-regulated surface determinants (Isd) of *Staphylococcus aureus*:
stealing iron from heme**

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*Committee on Microbiology, University of Chicago, Cummings Life Science Center Rm 607, 920 East 58th Street, Chicago, IL 60637, USA***Abstract**

Cell-wall sorted proteins of the *Staphylococcus aureus* iron-regulated surface determinant system bind human hemoproteins, remove the heme molecule, and transport heme through the cell wall and plasma membrane for accumulation in the bacterial cytoplasm. Once inside the cell, the porphyrin ring of heme is degraded by heme degrading monooxygenases, leading to the formation of free iron for use by the bacterium as a nutrient source.

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1. Introduction

Staphylococci are capable of causing a diverse array of diseases, ranging in severity from food poisoning and urinary tract infections to toxic shock syndrome, bacteremia, and septic endocarditis. Arguably the most important cause of life-threatening bacterial infections in the world, *Staphylococcus aureus* is the leading cause of nosocomial diseases in the United States, the most prevalent pathogen isolated from skin and soft-tissue infections [1], one of the four leading causes of food-borne illness [1], and one of the most common etiological agents of infectious endocarditis [1]. The recent identification of isolates of *S. aureus* resistant to the antibiotic vancomycin [2], considered the last line of defense against bacterial infection, underscores the importance of identifying novel therapeutic targets against this important emerging pathogen. One area that has received recent attention as a promising therapeutic target is bacterial iron acquisition systems. This is primarily due to the fact that, with some notable exceptions [3], virtually all bacterial pathogens require iron to infect.

Evolution has provided humans with a defense mechanism that exploits the strict iron requirement of bacterial

pathogens: iron is virtually unavailable inside the human host. Iron limitation is one of the most important innate human defenses against bacterial infection. In fact, iron is the only limiting nutrient to bacterial growth inside the human host [4]. Virtually all bacteria require iron in the range of 0.4–4.0 μM [5], which is well above the physiologic concentration of iron readily available inside humans [4]. Although plenty of iron exists inside the human body to support bacterial growth, evolution has provided humans with multiple methods of preventing bacteria from acquiring this essential nutrient. These include low solubility of iron at physiological pH [6], the intracellular location of iron [6], and the sequestration of this ion within iron-binding proteins such as the glycoproteins transferrin and lactoferrin, or heme-containing proteins (hemoproteins).

Transferrin is an extracellular iron carrier molecule that can be found in the serum and the lymph of humans. Transferrin has been reported to be a viable iron source for numerous pathogens, including *Bordetella pertussis*, *Haemophilus influenzae*, *Neisseria meningitidis*, *Pasteurella haemolytica*, and *S. aureus* [4]. It is surprising that considering the large body of existing literature describing bacterial use of transferrin as an iron source, transferrin comprises only 6–7.5% of the body's iron pool that is accessible to bacteria [7]. The majority of iron in the human body is found intracellularly bound to hemoproteins. Circulating erythrocytes are the primary iron-containing cells in the body, with between 60% and 75% of the body's iron content being bound to the oxygen transport protein, hemoglobin. The oxygen storage

Abbreviations: (Isd), Iron-regulated surface determinants; (GAPDH), glyceraldehyde-3-phosphate dehydrogenase.

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protein, myoglobin, accounts for an additional 8–10%. Other hemoproteins, along with iron-containing proteins such as iron sulfur proteins, make up a further 4–5% of iron in the human body [7]. Although all iron-containing proteins present in the host represent potential nutrient sources to bacteria, certain bacteria have co-evolved with humans to utilize the most abundant source of iron in their host, hemoproteins.

Porphyrins are the most colorful and most widely utilized enzyme cofactors in nature [8]. The porphyrin heme is not only a superb biocatalyst but is also an excellent iron source to both prokaryotes and eukaryotes. Heme is a circular molecule containing a single iron atom bound to four-ring nitrogen atoms of the macrocyclic porphyrin. Proteins that contain heme as a cofactor, or hemoproteins, are responsible for numerous functions in the cell, including oxygen carriage and storage, activation of molecular oxygen, and electron transport [8]. The most abundant hemoproteins in the body, hemoglobin and myoglobin, are responsible for carrying and storing oxygen, respectively. Free hemoglobin is not tolerated by the body, and therefore upon red blood cell lysis, hemoglobin dimers are bound by the hemoglobin carrier molecule, haptoglobin, and transported to the liver. Free heme is a highly reactive molecule that is also poorly tolerated by cells. When heme is liberated from hemoproteins, as occurs during erythrocyte lysis, liberated heme is bound by hemopexin, and the complex is rapidly cleared from the circulation and routed to the liver. The heme moieties of enzymes such as peroxidases, catalases, and cytochrome P₄₅₀s are responsible for the activation of oxygen or the oxidation of cellular molecules. Additionally, electron transport proteins, such as cytochromes *b*, utilize the iron atom of the heme molecule for the transfer of electrons. The variety and the abundance of hemoproteins in humans provide multiple potential iron sources to infectious microorganisms seeking this essential nutrient.

Although the analysis of iron-uptake systems has achieved considerable detail in Gram-negative pathogens, recent literature has benefited from a surge in the number of reports describing iron-uptake systems in Gram-positive bacteria. Siderophore [9] and transferrin receptor [10,11]-based iron acquisition systems in Gram-positive bacteria have been reported, and descriptions of heme and hemoprotein-uptake systems have also begun to emerge. Some examples of the latter include the identification of heme-binding proteins and uptake systems in *B. pertussis*, *B. bronchiseptica* [12] or *Streptococcus pyogenes* [13], and a detailed account of heme uptake and degradation in *Corynebacterium diphtheriae* [14]. In addition, identification of the iron-regulated surface determinant (Isd) system in *S. aureus* has demonstrated the importance of cell-wall sorted proteins in heme binding and transport [15]. To date, the Isd system comprises the only known heme-iron utilization pathway in *S. aureus*. This review seeks to describe what is currently known regarding iron acquisition in *S. aureus*, with an emphasis on how *S. aureus* utilizes the Isd system to access the most abundant iron source in the body, hemoprotein-bound heme-iron.

2. Iron uptake in *S. aureus*

2.1. Importance of iron to infection and growth of *S. aureus*

Although *S. aureus* can survive in fluids with iron concentrations as low as 0.04 μM [16], iron is vital to the survival of this pathogen. The importance of iron to staphylococcal infection is best outlined by early experiments describing human and animal *S. aureus* infection models. These experiments revealed that infection of human volunteers with *S. aureus* intradermally, subcutaneously, or directly into skin lesions did not result in significant disease. However, when sutures coated with staphylococci were surgically inserted into the skin, significant disease occurred within 24 h, presumably due to ischemia and necrosis introduced via the surgical procedure [4,17]. These findings led to the hypothesis that the ischemia and necrosis cause a decrease in *Eh* (oxidation reduction potential) and pH at the site of infection. Decreased *Eh* and pH would lead to a subsequent change in the oxidation state of the iron atoms at the infection site, i.e. the conversion of ferric ions to the more bioavailable ferrous form, thereby providing iron to the invading staphylococci. This hypothesis is supported by the observation that increased levels of ferrous iron and heme have been shown to stimulate the growth of *S. aureus* inside and outside of rabbit polymorphonuclear leukocytes in blood clots [18]. It is of note that staphylococcal growth acceleration was even more pronounced in the presence of exogenously added heme. These results suggest that *S. aureus* utilizes multiple iron sources during infection, that iron utilization is affected by the status of the infection site, and that iron availability can influence the outcome of infection. Multiple iron-uptake systems are deployed by *S. aureus* to ensure successful nutrient acquisition. *S. aureus* can acquire iron through siderophore-dependent mechanisms, which remove host iron from sources such as the labile iron pool, transferrin and lactoferrin, or the iron storage protein ferritin. In addition, *S. aureus* can use free heme and hemoproteins including hemoglobin, and hemoglobin-haptoglobin as iron sources [15,19]. It seems plausible that staphylococci employ specific iron transport systems, depending on the growth phase of the organism, the specific site of infection, the level of tissue damage at the site of infection, and the nature of the infected hosts.

2.2. *S. aureus* possesses a siderophore-dependent iron-uptake system

Siderophores are low-molecular-weight iron chelators produced by bacteria that bind iron with an extremely high affinity and are capable of removing iron from host proteins, for transport into the bacterial cell. At the surface of the bacterium, siderophores interact with their cognate receptors and transporters to bring iron into the cytoplasm for use as a nutrient source. *S. aureus* produces three different sidero-

phores, staphyloferrin A [20], staphyloferrin B [21], and aureochelin [22] and also possesses the ability to use heterologous hydroxamate siderophores [9]. Although clearly the most-studied bacterial iron-uptake systems, the relevance of siderophores to the infectious process has not been adequately described. Siderophore acquisition systems may have a dramatic role in staphylococcal pathogenesis, or they may represent a method of acquiring iron in non-pathogenic settings, such as during commensal colonization of the nasopharynx or skin.

2.3. The elusive transferrin receptor of *S. aureus*

Although *S. aureus* is capable of utilizing transferrin as a sole iron source, the precise mechanism by which this occurs is not known. The secretion of siderophores likely enables *S. aureus* to capture transferrin iron for uptake via siderophore receptors. This raises the possibility that *S. aureus* might not require a specific transferrin receptor. This theory is also fueled by the fact that the identity of a specific transferrin receptor protein is still under debate. Two groups have ascribed transferrin receptor properties to separate proteins. Modun and Williams [11] reported that the transferrin receptor protein, Tpn, is a multifunctional cell-wall glyceraldehyde-3-phosphate dehydrogenase (GAPDH). This observation was surprising, due to the lack of any obvious link between the GAPDH activity of this enzyme, the typical cellular location of GAPDH in the bacterial cytoplasm, and the absence of tight iron-regulation of GAPDH activity. More recently, Taylor and Heinrichs [10] disputed the role of GAPDH in transferrin binding and reported that *S. aureus* strains lacking the *gap* gene (encoding GAPDH) displayed wild-type levels of transferrin binding. Instead, it was proposed that another protein, named StbA, may function as a transferrin-specific receptor in *S. aureus* [10]. StbA, also known as IsdA, has been shown by Mazmanian et al. [15] to be responsible for the binding and uptake of heme-iron. Additional work is needed to determine whether or not *S. aureus* expresses a transferrin-specific receptor, as the ability of staphylococci to grow on transferrin iron may be due to the presence of siderophore-uptake pathways.

2.4. Staphylococci bind heme-iron and hemoproteins and utilize them as iron sources for growth

Staphylococci can utilize both heme and hemoproteins as a sole iron source [15,19]. In addition to transporting heme-iron into the bacterial cytoplasm, *S. aureus* also binds large amounts of [⁵⁵Fe]heme within the cell-wall envelope, a property that is not affected by heat killing of the bacteria. One simple explanation for this result is that appreciable amounts of [⁵⁵Fe]heme are bound to heme or hemoprotein-specific staphylococcal receptors without subsequent passage into the bacterial cytoplasm [15]. It is presumed that *S. aureus* encounters hemoproteins upon the lysis of red blood cells mediated by the secretion of numerous bacterial hemolysins.

The lytic properties of *S. aureus* toxins have long been viewed as a pathogenic strategy for the lysis of immune cells. However, erythrocyte lysis during infection certainly leads to the accumulation of hemoglobin, heme, hemoglobin–haptoglobin, and heme–hemopexin complexes in extracellular fluids. All of these proteins and protein complexes represent iron sources to invading staphylococci. Thus, one can view the staphylococcal Isd system as a pathogenic strategy of scavenging heme-iron during infection by tapping into the rich iron source of hemoproteins [15].

3. The Isd system of *S. aureus*

The Isd system of *S. aureus* forms a heme-uptake machinery composed of numerous genes encoded by five transcriptional units (*isdA*, *isdB*, *isdCDEFsrtBisdG*, *isdH*, and *isdI*) (Fig. 1A). The system encompasses genes that encode cell-wall anchored surface proteins (IsdABCH), a membrane transporter (IsdDEF), a sortase (SrtB), and two cytoplasmic proteins (IsdGI). The initial interaction of the Isd system with hemoproteins must occur at the level of the cell wall, the surface organelle of Gram-positive bacteria.

3.1. The Isd system of *S. aureus* is associated with sortases

The cell wall of Gram-positive bacteria is composed of a peptidoglycan macromolecule that serves multiple functions. In addition to providing protection against osmotic lysis, the peptidoglycan functions as a cytoskeletal element for the anchoring of surface proteins. Proteins can be immobilized to the cell wall of Gram-positive bacteria through covalent and non-covalent linkages. A large number of surface proteins are covalently anchored to the cell wall peptidoglycan by a mechanism requiring a C-terminal sorting signal with an LPXTG motif [23]. Sortases, membrane anchored transpeptidases, cleave surface proteins at the LPXTG motif and catalyze the formation of an amide linkage between the C-terminal end of the polypeptide chain and the cell wall crossbridge of peptidoglycan. The first sortase identified in Gram-positive bacteria was found in *S. aureus* and is known as sortase A [24]. Sortase A (*srtA*) is responsible for the cell-wall anchoring of at least 20 proteins involved in various processes, including nutrient acquisition, ligand binding, and immune system evasion [23]. Three of the cell-wall sorted proteins of the Isd system are anchored to the cell wall by sortase A (IsdA, IsdB, and IsdH). Analysis of the *S. aureus* genome for *srtA* homologs revealed a second sortase gene, *srtB* [25]; *srtB*, encoding for sortase B, is located in the transcriptional unit *isdCDEFsrtBisdG* [25]. Sortase B is also a component of Isd, as the transpeptidase is involved in anchoring the heme-binding protein IsdC to the cell-wall envelope. This is a unique demonstration of two separate sortases responsible for cell-wall anchoring of various components of a single nutrient acquisition system.

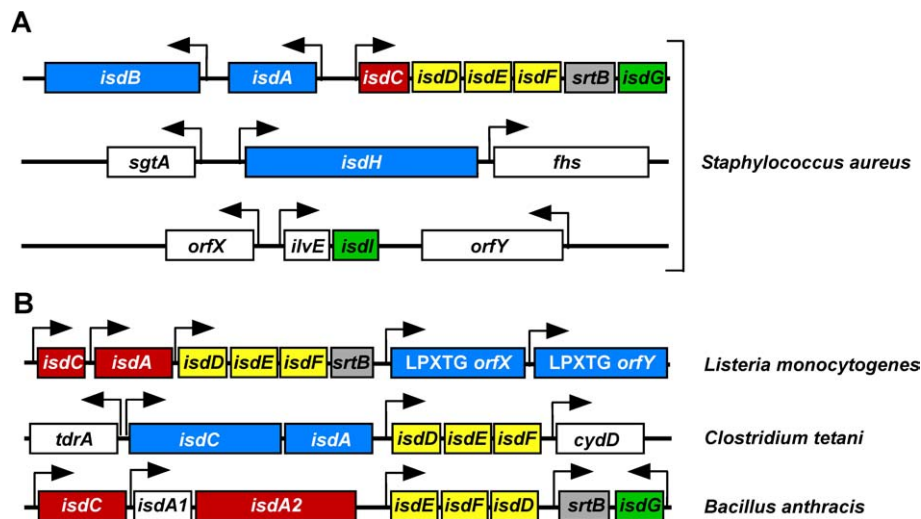


Fig. 1. Schematic representation of the genomic regions encoding Isd system components in various Gram-positive pathogens. (A) The Isd system of *S. aureus*. The three lines represent three distinct genomic regions of the *S. aureus* chromosome. (B) The Isd system of *L. monocytogenes*, *Clostridium tetani*, and *B. anthracis*. Arrows represent the predicted direction of transcription. The genes are color coded, based on their classifications: blue represents genes predicted to code for sortase A substrates, red represents genes predicted to code for sortase B substrates, yellow genes are predicted to produce proteins responsible for the formation of heme transport systems across the plasma membrane, gray genes are predicted to encode for sortases, and green genes are predicted to encode for heme-degrading enzymes. The genes shown in white are uncharacterized; however, they are named after their closest identified homolog. Genes without significant identity to any known gene are designated *orfX* or *orfY*.

3.2. The Isd system transports heme-iron into the cell

The Isd system is so named based on the iron regulation of all genes in the cluster, and the observation that four of the protein products of the system (IsdA, IsdB, IsdC, and IsdH) are cell-wall anchored. Heme-binding experiments revealed that all Isd proteins exhibit some level of heme binding, with the cytoplasmic proteins appearing to bind heme with the strongest affinity [15]. The heme-binding property of Isd proteins is exemplified upon purification of recombinant proteins from *Escherichia coli*, whereupon Isd proteins complex endogenous heme and therefore exhibit a red hue due to the colored nature inherent to heme–protein complexes. Inactivation of individual components of the Isd system does not abolish the ability of *S. aureus* to utilize heme-iron as a nutrient source for growth, implying that additional heme-uptake systems exist in this organism [15]. However, inactivation of *isdA* leads to a decrease in the amount of radiolabeled heme-iron that associates with intact staphylococcal cells. Furthermore, inactivation of *isdA*, encoding for a cell-wall sorted protein, or *isdF*, encoding for the permease component of the heme membrane transport system, decreases the amount of radiolabeled heme-iron that enters the staphylococcal cytoplasm [15]. Together, these results suggest that the Isd system is responsible for binding and uptake of heme-iron in *S. aureus*.

3.3. Unique hemoprotein receptors encoded in the Isd system

IsdB binds hemoglobin with qualities consistent with a receptor–ligand interaction, and likely represents a staphylococcal hemoglobin receptor [15]. An intrachromosomal

paralog to *isdB* exists in *S. aureus*, encoding a protein predicted to possess 85% amino acid identity to IsdB [15]. This gene, originally named *isdH*, is located at a chromosomal locus outside of the main Isd cluster (Fig. 1A), and contains an LPXTG motif, implying that it is sorted to the cell wall by sortase A [15]. Investigations into IsdH, also known as HarA, have revealed that this protein is expressed during human infections and is immunogenic [26]. In addition, IsdH/HarA was found to bind to the β subunit of human haptoglobin, and to be Fur regulated [19]. IsdH/HarA contains two similar regions, named D1 and D2, that are each sufficient for haptoglobin and haptoglobin–hemoglobin-binding activity. Finally, IsdH does not possess any significant homology to previously identified haptoglobin or haptoglobin–hemoglobin receptors, and hence represents a member of a novel hemoprotein receptor class [19]. The presence of multiple cell-wall sorted proteins with unique hemoprotein ligands in *S. aureus* suggests a complex heme-iron utilization system composed of numerous receptor–ligand interactions. These observations begin to explain the drastic phenotype seen in heme-iron utilization assays upon inactivation of the staphylococcal sortase genes.

4. The role of sortases in heme-iron uptake

4.1. Two separate sortases anchor the heme-uptake system to the cell wall

IsdA, IsdB, and IsdH are LPXTG motif-containing protein components of the heme-uptake system, and require sortase A for cell-wall anchoring. IsdC may be the only substrate of sortase B in *S. aureus*, as bioinformatics analysis

failed to identify other surface protein genes with an NPQTN motif sorting signal [25]. This implies that in *S. aureus*, sortase B exists to anchor an individual component of the heme-uptake system to the cell wall. Sortase A is constitutively active; however, the expression of *isdA*, *isdB*, and *isdH* are under the control of the iron-dependent repressor Fur [15,19]. *srtB*, which is in the same transcriptional unit as *isdC*, is also regulated by Fur, preventing the transcription of *isdC* and *srtB* under iron replete conditions. This level of regulation ensures that SrtB, IsdA, IsdB, IsdC, and IsdH are only produced when the organism is in need of iron. The apparent reason that *S. aureus* possesses two separate sortases involved in anchoring a single uptake system may be due to individual Isd proteins having unique cell-wall localization sites. IsdA, IsdB, and IsdH are displayed on the surface of the bacterial cell anchored to cross-linked murein subunits. In contrast, IsdC is buried within the bacterial cell wall, and does not appear to be tethered to cross-linked murein [15], suggesting that IsdC is attached to a unique portion of the cell wall compared with other protein components of the Isd system. It is known that sortase A substrates are anchored to the pentaglycine crossbridge of the staphylococcal cell wall; however, the anchoring point of sortase B sorted proteins has not yet been determined. The identification of the IsdC cell-wall anchor structure will provide much needed insight into the significance of two distinct sortase enzymes in *S. aureus* heme-iron acquisition.

4.2. Inactivation of staphylococcal sortases decreases heme-uptake and virulence

As one might expect, the inability to covalently anchor a large subset of proteins to the cell wall significantly affects bacterial virulence. Inactivation of *srtA* in *S. aureus* markedly reduces virulence in mouse models of acute infection, renal abscess, and arthritis [23,27]. The pleiotropic effect that inactivation of *srtA* has on numerous bacterial virulence factors potentially explains this phenotype. However, it is interesting to note that approximately 15% of all LPXTG-containing proteins identified in *S. aureus* are members of the Isd system. This allows us to speculate that inability to bind host hemoproteins is responsible for the dramatic virulence defect seen in a *srtA* mutant strain. In support of this hypothesis, staphylococcal *srtA* mutants are not able to use heme-iron as a sole iron source for growth. Further, inactivation of *srtA* reduces [⁵⁵Fe]heme co-sedimentation with intact staphylococci, and also reduces [⁵⁵Fe]heme transport into the cytoplasm [15]. The specific role of heme-iron utilization in pathogenesis needs to be tested by investigating the virulence defect caused by inactivation of *isdA*, *isdB*, or *isdH* individually and collectively. Elimination of *srtB* does not affect virulence as drastically as a *srtA* mutation; however, mice infected with *srtB* mutant strains showed better survival rates than mice infected with wild-type *S. aureus* in the acute mouse model of infection [27]. Also, sortase B is required for persistent infection, as demonstrated in a murine renal ab-

scence model [25]. Furthermore, staphylococci lacking *srtB* display defects in the ability to establish murine arthritis [27]. It is presumed that the virulence defect of the *srtB* mutant strain is due to an inability to acquire heme-iron from the host through the loss of IsdC anchoring. This is supported by the growth defect of *srtB* mutants on heme-iron as a sole iron source, and decreased binding and transport of radiolabeled heme into staphylococci upon inactivation of *srtB* [15]. The observation that inactivation of *srtB* leads to a decreased ability of *S. aureus* to persist in tissues implies that heme provides bacteria with enough iron to multiply in the otherwise iron-free environment of the host after bacterial iron stores have been exhausted.

5. Heme degradation in the bacterial cytoplasm

In order for bacterial pathogens to utilize heme as an iron source, the microbes must be able to access the iron atom contained within the porphyrin ring. To date, the only enzymes identified capable of degrading the porphyrin ring of heme belong to a family of monooxygenases known as heme oxygenases. Heme oxygenases are ubiquitous in nature, and in the presence of electron donor, are responsible for the degradation of heme to biliverdin, carbon monoxide, and iron [28]. In mammals, heme oxygenases are primarily involved in protecting cells against the toxic affects of heme, whereas bacteria utilize heme oxygenases to access the iron atom of heme for use as a nutrient source [29]. A search of all complete and incomplete bacterial genomes reveals that heme oxygenase homologs can be identified in five bacterial genera, including *Corynebacterium*, *Neisseria*, *Pseudomonas*, *Agrobacterium* and *Streptomyces* [30]. The paucity of identifiable heme-degrading enzymes in bacterial pathogens is interesting considering that dozens of disease-causing microbes are capable of utilizing heme as a sole iron source for growth [4]. The lack of identifiable members of the only known heme-degrading enzyme family in the genomes of bacterial pathogens suggests the existence of additional, as yet unidentified, enzymes capable of degrading heme.

5.1. *IsdG* and *IsdI* encode for heme-degrading enzymes in the staphylococcal cytoplasm

The gene encoding IsdG is the terminal gene in the transcriptional unit, *isdCDEFsrtBisdG* (Fig. 1A). An intrachromosomal paralog to *isdG*, exhibiting approximately 70% identity to *isdG*, exists in *S. aureus* and is known as *isdI*. The predicted protein products of IsdG and IsdI do not have significant similarity to any previously identified heme-degrading enzyme, and encode for cytoplasmic proteins belonging to a family of monooxygenases [30]. IsdG and IsdI are iron-regulated and capable of binding hemin with characteristics consistent with known heme-degrading enzymes. Furthermore, incubation of either IsdG or IsdI with hemin in the presence of a suitable electron donor leads to the degra-

dation of heme to biliverdin and free iron for use by *S. aureus* as a nutrient source [30]. Expression of IsdI in a *Corynebacterium ulcerans* heme oxygenase-deficient mutant strain complements the heme utilization defect of this strain, demonstrating an in vivo heme degradation activity for this enzyme [30]. Taken together, these results reveal that IsdG and IsdI represent members of a heme-degrading enzyme family in *S. aureus*.

5.2. IsdG and IsdI are members of a novel family of heme-degrading enzymes in Gram-positive pathogens

Although previously identified heme oxygenases do not share tremendous sequence identity with each other, they are all predicted to possess a conserved monomeric alpha helical folding pattern as evidenced by the crystal structures of mammalian and bacterial heme oxygenases [29]. In addition, a G-X-X-X-G motif, and a conserved proximal histidine residue are modeled to be required for catalytic activity [29]. IsdG and IsdI do not possess either of these conserved amino acid patterns. This observation, combined with the lack of any identifiable sequence conservation between IsdG or IsdI and previously identified heme oxygenases implies that these enzymes are members of a novel heme-degrading enzyme family. Determination of the crystal structure of either member of this enzyme family is needed to determine if IsdG and IsdI possess the conserved alpha-helical folding pattern seen in other heme oxygenases. Homologs to IsdG and IsdI can be identified in numerous Gram-positive pathogens, including *Bacillus anthracis*, *Staphylococcus epidermidis*, and *Listeria monocytogenes*, implying that this pathway of heme degradation is conserved in Gram-positive pathogens.

6. A conserved method of heme-iron uptake in Gram-positive pathogens

Iron-regulated surface determinant systems can be identified in *B. anthracis*, *B. halodurans*, *B. cereus*, *L. monocytogenes*, *L. innocua*, and *Clostridium tetani* (Fig. 1B). The absence of an intact Isd system in *S. epidermidis* implies that the pathogenic staphylococci utilize different methods of scavenging iron from the host during colonization and infection. Interestingly, comparison of the cell-wall sorted proteins in the Isd systems reveals that homologous proteins are anchored by different sortases, depending on the organism. For example, IsdA homologs contain LPXTG motifs in *S. aureus* and *C. tetani*; however, in *B. anthracis* and *L. monocytogenes*, the IsdA homologs harbor NPQTN motifs, implying sortase B-dependent anchoring. Also, IsdC homologs from *S. aureus*, *B. anthracis*, and *L. monocytogenes* are NPQTN motif-containing SrtB substrates, while the *C. tetani* IsdC homolog possesses an LPXTG motif, implying sortase A-mediated cell-wall anchoring. This suggests that the evolution of heme acquisition in Gram-positive pathogens has provided each bacterial species with the ap-

propriate sortase-substrate combination to best suit their host niche. Further evidence for evolutionary modification in the heme-uptake systems of Gram-positive pathogens can be identified upon analysis of the individual Isd systems. The Isd systems of *S. aureus* and *B. anthracis* are closely linked to genes predicted to encode sortase enzymes (*srtB*) and heme-degrading enzymes (*isdG*) (Fig. 1), demonstrating a genomic linkage to heme-uptake, cell-wall sorting of hemoprotein receptors, and heme degradation. In contrast, the genes of the *C. tetani* Isd system are located adjacent to genes predicted to encode a cytochrome synthesis system (*cydD*). This implies that instead of heme degradation through IsdG or IsdI homologs, *C. tetani* may incorporate intact host heme directly into bacterial heme-containing proteins.

7. Conclusions

Based on the existing data in *S. aureus*, a model for the Isd-mediated acquisition of heme-iron from hemoproteins begins to emerge. We presume that upon infection of vertebrate hosts, *S. aureus* expresses toxins capable of causing considerable damage to host cells, including the lysis of erythrocytes. This lysis of red blood cells will lead to the release of numerous molecules of hemoglobin. More specifically, either free hemoglobin or heme that has been released from hemoglobin, or hemoglobin-haptoglobin and heme-hemopexin complexes will become available. Due to the iron-restrictive environment of the human host, Fur-mediated repression of the Isd system will be relieved, resulting in expression and assembly of the Isd system within the cell-wall envelope. This will allow for hemoprotein ligands to be bound by the staphylococcal cell-wall-associated hemoprotein receptors IsdA, IsdB, and IsdH. It is also conceivable that other heme-binding serum proteins such as albumin, heme-containing cellular proteins such as cytochromes, catalase, and peroxidase, or the heme-containing oxygen storage protein myoglobin are recognized by the Isd system as iron sources. Once attached to specific hemoprotein receptors, heme-iron is somehow removed from the hemoprotein and transported across the cell wall in an IsdC-dependent manner. The hypothesis that IsdC is involved in heme transport through the cell wall is primarily based on the observation that IsdC is sorted to a unique portion of the cell wall. It is possible that IsdC is another hemoprotein receptor for which a specific ligand has not yet been identified; however, the observation that the entire IsdC protein is buried in the cell wall [15] makes this hypothesis unlikely. Upon passage through the cell wall, heme-iron is transported by IsdDEF across the plasma membrane into the bacterial cytoplasm. Once inside the cytoplasm, heme is degraded to biliverdin, carbon monoxide, and free iron for use by the bacterium as a nutrient source (Fig. 2). The fate of biliverdin and carbon monoxide in the bacterial cell is not yet known; however, in eukaryotes, these molecules have important roles in protection against oxidative stress and intracellular signaling. Al-

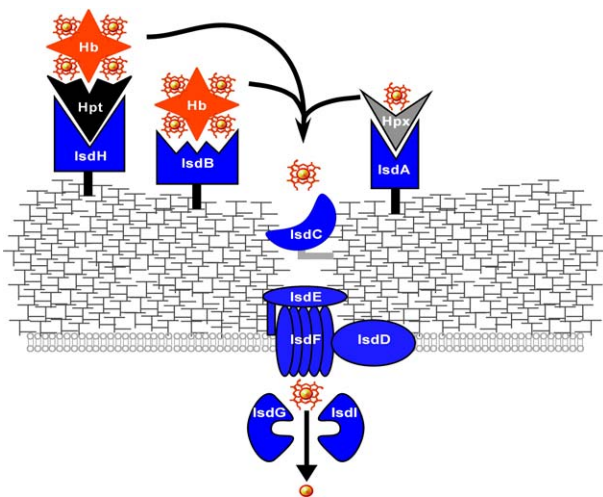


Fig. 2. A model for Isd-mediated heme-iron transport and utilization by *S. aureus*. IsdA, IsdB, and IsdH are sorted to the cell wall by sortase A and are potential receptors to hemoprotein ligands released upon erythrocyte lysis, including haptoglobin (Hpt), hemoglobin (Hb), and hemopexin (Hpx). In addition, red blood cell destruction will result in the formation of free heme, which may serve as a ligand to the hemoprotein receptors of the Isd system. Upon binding to the Isd receptors, heme is released from the hemoproteins by an as-yet-undefined mechanism, and passed through the cell wall in an IsdC-dependent manner. The heme molecule is then transported through the membrane transport system composed of IsdDEF into the cytoplasm. Upon entry into the cytoplasm, heme is degraded by IsdG and IsdI acting as heme monoxygenases. This leads to the release of free iron for use by the bacterium as a nutrient source.

though recent studies revealed the contours of a complex hemoprotein utilization pathway in Gram-positive bacteria, much work needs to be done to elucidate the mechanisms of heme extraction from hemoproteins, the mechanism of heme transport, the nature of specific hemoproteins recognized by the Isd system, and the heme-degrading characteristics of the enzymes within the Isd system. In addition, a comparison of the function of the Isd system across pathogens will provide valuable information regarding the conservation of heme-uptake systems across genera. A complete description of the heme-uptake systems of Gram-positive pathogens will provide much needed insight into a process vital to survival of this group of organisms which contains numerous emerging threats to human health.

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References

- [1] J.J. Weems Jr, The many faces of *Staphylococcus aureus* infection. Recognizing and managing its life-threatening manifestations, *Postgrad. Med.* 110 (4) (2001) 24–26 29–31, 35–36.
- [2] M.J. Rybak, R.L. Akins, Emergence of methicillin-resistant *Staphylococcus aureus* with intermediate glycopeptide resistance: clinical significance and treatment options, *Drugs* 61 (1) (2001) 1–7.
- [3] J.E. Posey, F.C. Gherardini, Lack of a role for iron in the Lyme disease pathogen, *Science* 288 (5471) (2000) 1651–1653.
- [4] J.J. Bullen, E. Griffiths, *Iron and Infection: Molecular, Physiological and Clinical Aspects*, John Wiley and Sons, New York, 1999.
- [5] E.D. Weinberg, Iron and infection, *Microbiol. Rev.* 42 (1) (1978) 45–66.
- [6] K.R. Bridges, P.A. Seligman, in: T.P. Stossel, R.I. Handlin, S.E. Lux (Eds.), *Disorders of Iron Metabolism in Blood, Principles and Practices of Hematology*, J.P. Lippencott Company, London, 1995, pp. 1433–1472.
- [7] R. Crichton, *Inorganic Biochemistry of Iron Metabolism: From Molecular Mechanisms to Clinical Consequences*, 2, John Wiley & Sons, Ltd., England, West Sussex, 2001.
- [8] I. Stojiljkovic, D. Perkins-Balding, Processing of heme and heme-containing proteins by bacteria, *DNA Cell Biol.* 21 (4) (2002) 281–295.
- [9] M.T. Sebulsky, D. Hohnstein, M.D. Hunter, D.E. Heinrichs, Identification and characterization of a membrane permease involved in iron-hydroxamate transport in *Staphylococcus aureus*, *J. Bacteriol.* 182 (16) (2000) 4394–4400.
- [10] J.M. Taylor, D.E. Heinrichs, Transferrin binding in *Staphylococcus aureus*: involvement of a cell wall-anchored protein, *Mol. Microbiol.* 43 (6) (2002) 1603–1614.
- [11] B. Modun, P. Williams, The staphylococcal transferrin-binding protein is a cell wall glyceraldehyde-3-phosphate dehydrogenase, *Infect. Immun.* 67 (3) (1999) 1086–1092.
- [12] C.K. Vanderpool, S.K. Armstrong, The *Bordetella bhui* locus is required for heme iron utilization, *J. Bacteriol.* 183 (14) (2001) 4278–4287.
- [13] B. Lei, L.M. Smoot, H.M. Menning, J.M. Voyich, S.V. Kala, F.R. Deleo, S.D. Reid, J.M. Musser, Identification and characterization of a novel heme-associated cell surface protein made by *Streptococcus pyogenes*, *Infect. Immun.* 70 (8) (2002) 4494–4500.
- [14] E.S. Drazek, C.A. Hammack, M.P. Schmitt, *Corynebacterium diphtheriae* genes required for acquisition of iron from haemin and haemoglobin are homologous to ABC haemin transporters, *Mol. Microbiol.* 36 (1) (2000) 68–84.
- [15] S.K. Mazmanian, E.P. Skaar, A.H. Gaspar, M. Humayun, P. Gornicki, J. Jelenska, A. Joachmiak, D.M. Missiakas, O. Schneewind, Passage of heme-iron across the envelope of *Staphylococcus aureus*, *Science* 299 (5608) (2003) 906–909.
- [16] D. Trivier, R.J. Courcol, Iron depletion and virulence in *Staphylococcus aureus*, *FEMS Microbiol. Lett.* 141 (2–3) (1996) 117–127.
- [17] S.D. Elek, P.E. Conen, The virulence of *Staphylococcus pyogenes* for man; a study of the problems of wound infection, *Br. J. Exp. Pathol.* 38 (6) (1957) 573–586.
- [18] G.P. Gladstone, E. Walton, The effect of iron and haematin on the killing of staphylococci by rabbit polymorphs, *Br. J. Exp. Pathol.* 52 (5) (1971) 452–464.
- [19] A. Dryla, D. Gelbmann, A. Von Gabain, E. Nagy, Identification of a novel iron regulated staphylococcal surface protein with haptoglobin-haemoglobin binding activity, *Mol. Microbiol.* 49 (1) (2003) 37–53.
- [20] S. Konetschny-Rapp, G. Jung, J. Meiwes, H. Zahner, Staphyloferrin A: a structurally new siderophore from staphylococci, *Eur. J. Biochem.* 191 (1) (1990) 65–74.
- [21] H. Drechsel, S. Freund, G. Nicholson, H. Haag, O. Jung, H. Zahner, G. Jung, Purification and chemical characterization of staphyloferrin B, a hydrophilic siderophore from staphylococci, *Biometals* 6 (3) (1993) 185–192.
- [22] R.J. Courcol, D. Trivier, M.C. Bissinger, G.R. Martin, M.R. Brown, Siderophore production by *Staphylococcus aureus* and identification of iron-regulated proteins, *Infect. Immun.* 65 (5) (1997) 1944–1948.
- [23] S.K. Mazmanian, H. Ton-That, O. Schneewind, Sortase-catalyzed anchoring of surface proteins to the cell wall of *Staphylococcus aureus*, *Mol. Microbiol.* 40 (5) (2001) 1049–1057.

- [24] S.K. Mazmanian, G. Liu, H. Ton-That, O. Schneewind, *Staphylococcus aureus* sortase, an enzyme that anchors surface proteins to the cell wall, *Science* 285 (5428) (1999) 760–763.
- [25] S.K. Mazmanian, H. Ton-That, K. Su, O. Schneewind, An iron-regulated sortase anchors a class of surface protein during *Staphylococcus aureus* pathogenesis, *Proc. Natl. Acad. Sci. USA* 99 (4) (2002) 2293–2298.
- [26] H. Etz, et al., Identification of in vivo expressed vaccine candidate antigens from *Staphylococcus aureus*, *Proc. Natl. Acad. Sci. USA* 99 (10) (2002) 6573–6578.
- [27] I.M. Jonsson, S.K. Mazmanian, O. Schneewind, T. Bremell, A. Tarkowski, The role of *Staphylococcus aureus* sortase A and sortase B in murine arthritis, *Microbes Infect.* 5 (9) (2003) 775–780.
- [28] M.D. Maines, The heme oxygenase system: a regulator of second messenger gases, *Annu. Rev. Pharmacol. Toxicol.* 37 (1997) 517–554.
- [29] A. Wilks, Heme oxygenase: evolution, structure, and mechanism, *Antioxid. Redox Signal* 4 (4) (2002) 603–614.
- [30] E.P. Skaar, A.H. Gaspar, O. Schneewind, IsdG and IsdI, heme degrading enzymes in the cytoplasm of *Staphylococcus aureus*, *J. Biol. Chem.* 279 (2004) 436–443.