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Siderophore–drug complexes: potential medicinal applications of the 'Trojan horse' strategy

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The ability of bacteria to develop resistance to antimicrobial agents poses problems in the treatment of numerous bacterial infections. One method to circumvent permeability-mediated drug resistance involves the employment of the 'Trojan horse' strategy. The Trojan horse concept involves the use of bacterial iron uptake systems to enter and kill bacteria. The siderophore–drug complex is recognized by specific siderophore receptors and is then actively transported across the outer membrane. The recently identified benefits of this strategy have led to the synthesis of a series of siderophore-based antibiotics. Several studies have shown that siderophore–drug conjugates make it possible to design antibiotics with improved cell transport and reduce the frequency of resistance mutants. Growing interest in siderophore–drug conjugates for the treatment of human diseases including iron overload, cancer, and malaria has driven the search for new siderophore–drug complexes. This strategy may have special importance for the development of iron oxide nanoparticle-based therapeutics.

Antimicrobial therapy

Antibiotic resistance in bacteria is a growing health problem for all the major classes of antibiotics used in the treatment of infectious diseases. One of the crucial mechanisms of antibiotic resistance in bacteria is decreased permeability of the outer membrane. One method to circumvent the resistance caused by this mechanism is to 'smuggle' the antibiotic molecule into the bacterial cell by linking this molecule to a siderophore molecule [1–3] which is typically used as an iron chelator.

Bacteria have developed two systems of iron transport which are activated depending on the environmental conditions. The first system, termed the low-affinity system, is activated under conditions of relative abundance of iron; in other words, when the iron concentration is at least 10^{-5} M. This mechanism involves free diffusion of iron ions across cell membranes. The second mechanism, termed the high-affinity system, is active under stress conditions caused by iron deficiency in the environment of the bacterium and involves active transport of iron ions into the bacterial cells using siderophores [4]. Siderophores are low molecular weight iron-chelating compounds (usually less than 1 kDa). They are synthesized and secreted by many microorganisms under iron-limited conditions such as during invasion of the mammalian host by bacterial pathogens. More than 500 siderophores have been characterized so far in bacteria, fungi, and plants [5,6]. These compounds show high specificity and affinity for iron ($K_{aff} > 10^{30}$) and can scavenge iron away from iron-binding proteins in the host.

Great hopes were raised in recent years when it was found that sideromycins could act as potential drugs for the treatment of bacterial infections. Sideromycins are naturally occurring Fe^{3+} siderophores that are covalently linked to an antibiotic moiety. Examples of sideromycins include albomycins, salmycins, and ferrimycins. Sideromycins are delivered into bacterial cells using the siderophore-mediated iron uptake system, thus relying on a 'Trojan horse' strategy. The Trojan horse strategy is a promising method of delivering drug molecules to targeted sites – drugs or drug-like molecules (as conjugates) are attached to siderophores that are substrates for natural transport mechanisms. These siderophore–antibiotic conjugates are recognized by specific membrane receptors and are actively transported across the bacterial outer and cytoplasmic membranes. The iron is released from the siderophore–iron complex and is incorporated into heme and non-heme iron proteins [1,5,7,8].

Glossary

CECaT: cyclophosphamide, etoposide, carboplatin, and thiotepa.
CHOP: cyclophosphamide–adriamycin–vincristine–prednisone.
FepA: outer-membrane receptor from *E. coli* for ferric enterobactin.
FecA: outer-membrane ferric dicitrate receptor of *E. coli.*
FhuA: outer-membrane receptor from *E. coli* for ferric ferrichrome.
FhuD: periplasmic binding proteins from *E. coli.*
FptA: outer-membrane receptor from *P. aeruginosa* for ferric pyochelin.
FpvA: outer-membrane receptor from *P. aeruginosa* for ferric pyoverdin.
TonB protein complex: cytoplasmic transmembrane complex, consisting of the proteins TonB, ExbB, and ExbD, that spans the periplasm. TonB, ExbB, and ExbD are cytoplasmic membrane proteins of the energy-transducing protein complex.

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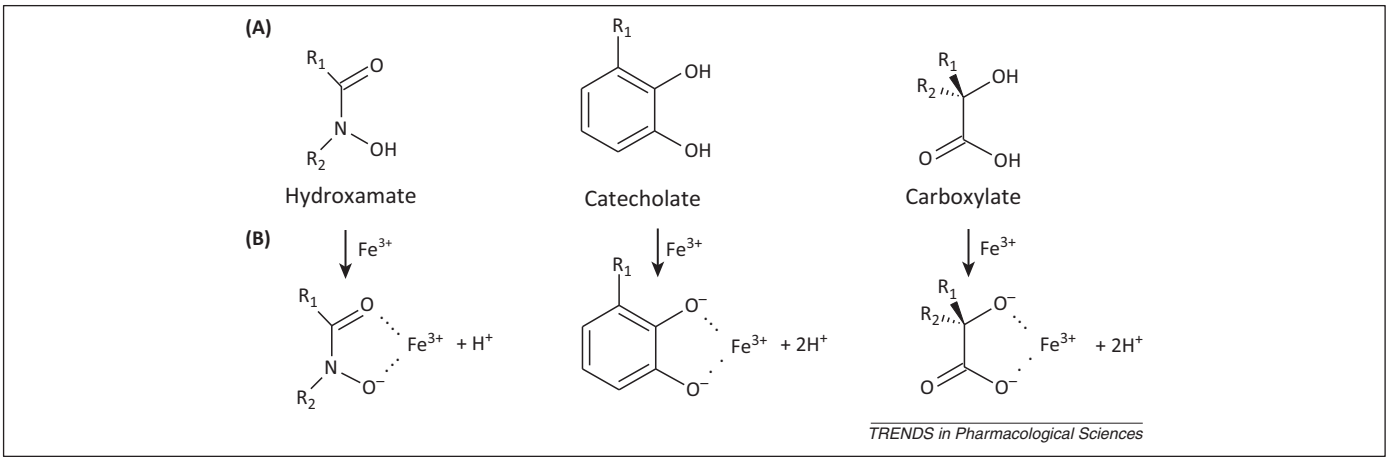

Figure 1. **(A)** The main structural component of siderophores that is responsible for iron coordination. **(B)** Complexes of the main functional groups of siderophores with iron (Fe^{3+}).

Siderophores are a highly diverse group of compounds, but the most common siderophores fall into three main classes based on the chemical nature of the moieties donating the oxygen ligands for Fe^{3+} coordination. These three classes are: catecholates, hydroxamates, and α-hydroxycarboxylates (Figure 1) [1,8–10]. Other less-common iron-binding moieties in siderophores are the hydroxyphenyloxazolone, α-aminocarboxylate, and α-hydroxyimidazole moieties [6]. Most siderophores are oxygen-donor, hexadentate ligands that form octahedral complexes with iron [10]. As hexadentate ligands, they coordinate Fe^{3+} ions to form 1:1 Fe^{3+}–siderophore complexes [11,12]. All of these liganding groups, namely catecholates, hydroxamates, and α-hydroxycarboxylates, form complexes with Fe^{3+} ion that are characterized by high thermodynamic stability. These three moieties form five-membered chelate rings and occupy two sites of the iron center. Siderophores can also form complexes with other metal ions such as Al^{3+}, Zn^{2+}, Ga^{3+}, Cr^{3+}, Pu^{3+}, and Pu^{4+} [9,10]. This is due to the presence of oxygen donors in the binding groups (functional groups of the siderophores) which show high affinity for other heavy metal ions [11].

In this review we show that knowledge regarding the structure of various siderophores, and the presence of microbial membrane receptors involved in the uptake of iron from these iron–siderophore conjugates, has opened new pathways in medical and pharmaceutical sciences. We also discuss whether – by analogy to the siderophore–drug complexes – it is possible to use these types of compounds to facilitate elimination of iron ions from the body. If such a system could be developed, it would offer a new approach to disorders of excess iron, one that could also solve the troublesome issue of the unclear fate of magnetic particles and the iron ions released from them.

Transport of iron–siderophore complexes in Gram-negative bacteria

Bacterial uptake of iron–siderophore complexes depends on outer-membrane receptors (OMRs), periplasmic binding proteins (PBPs), the TonB complex (see Glossary), and ABC-type transporters [13]. The first target for the iron–siderophore complex is the specific OMR which recognizes and binds the complex. The crystal structures of the outer-membrane receptors FepA, FhuA, and FecA from *Escherichia coli*, and of FpvA and FptA from *Pseudomonas aeruginosa*, show similarities in overall structure with the presence of similar domains. Active transport of the iron–siderophore complex across the outer membrane is an energy-dependent process [5,8,14]. The energy is provided by the TonB complex which comprises three cytoplasmic membrane proteins: TonB, ExbB, and ExbD. The proteins of the TonB complex transduce the proton motive force of the cytoplasmic membrane to the outer membrane to promote the active transport of the iron–siderophore complex across the outer membrane to the cytosol (Figure 2) [9,13,15,16]. Direct contact between the C-terminal domain of the TonB protein and the N-terminal domains of the OMR is required for energy transduction. The iron–siderophore complex is then actively transported into the periplasm and is recognized by and bound to its cognate periplasmic binding proteins (PBPs). The resulting iron–siderophore–PBP complex is transported across the cytoplasmic membrane by an ATP-binding cassette (ABC) transporter into the bacterial cytoplasm. ABC transporters are located in the inner membrane and utilize the energy of ATP hydrolysis to pump their substrates out of the cytoplasm against a concentration gradient [13,17,18]. Once siderophores reach the cytoplasm, iron is released from them by one of two mechanisms. The major iron release pathway involves reduction of siderophore-bound Fe^{3+} to Fe^{2+} by iron reductase and its subsequent transfer to various acceptor molecules within the cell membrane and/or cell interior. The other mechanism of iron release involves hydrolysis of the iron–siderophore complex, which requires specific enzymes. The iron-free siderophore is degraded or secreted by efflux pumps (Figure 2) [1,5,9,13].

In contrast to Gram-negative bacteria, Gram-positive bacteria do not possess an outer membrane or periplasmic zone, and the transport of the iron–siderophore complex into the cytoplasm may therefore differ from that in Gram-negative bacteria. The cell wall of Gram-positive bacteria is composed of 40 layers of murein. Chemically, about 30–70% of the dry mass of the wall is accounted for by peptidoglycan. Peptidoglycan contains teichoic and