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Biochemical Insights into Siderophore Esterases

Dongyue Yan, Yidao Jiang*

Department of Otolaryngology, Jingzhou Hospital Affiliated to Yangtze University, Jingzhou, China
Email: *1069670346@qq.com

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Abstract

Iron is an essential but excessively toxic nutrient. Although iron is rich in nature, the acquisition of iron is a challenge to life. Its solubility is very low because it is mostly in the form of oxidation or hydroxide. In order to overcome this, microorganisms have evolved a variety of iron absorption pathways, the most important of which is the siderophore-dependent iron absorption pathway. Both bacteria and fungi require specific siderophore esterases to encourage the release of iron within the cell. A deeper understanding of siderophore esterases is crucial for the development of new antibacterial and antifungal diagnostic and therapeutic approaches. There have been many recent studies on anti-infectives via siderophore antibiotic couplers in which siderophore esterases have also played an important role, and in this review, we provide an overview of several of the more common iron carriers as well as siderophore esterases in terms of structure as well as function.

Keywords

Siderophore, Siderophore Esterase, Hydrolysis, Enterobactin, Antibiotic

1. Introduction

Iron is an indispensable element for the growth and virulence of almost all pathogenic microorganisms, and acts as a catalyst in oxygen metabolism, electron transfer, amino acid metabolism, DNA and RNA synthesis, and mutual interaction [1]. Although iron is the fourth largest element in the earth's crust, its solubility is very low because it is mostly in the form of oxidation or hydroxide. The concentration of free iron ions in the environment is much lower than that required for microbial growth [2]. As a result, microbes have evolved sophisticated mechanisms for the uptake and storage of iron, such as the production of side-

*Corresponding author.

rophore (high affinity and low molecular weight metal chelating agents). The activity of specific hydrolases promotes the release of heavy metal atoms in cells. The discovery and clinical application of antibiotics have saved countless lives. However, because of the misuse of antibiotics, many pathogens have developed drug resistance, and human beings are about to face the situation of having no drugs available, so the research related to antibiotic resistance has attracted extensive attention from society and researchers [3] [4] [5].

2. Types of Siderophores

According to the difference of chemical properties, siderophores can be divided into three types: hydroxamate siderophores, catecholate siderophores and carboxylate siderophores.

2.1. Hydroxamate Siderophores

Hydroxamate siderophores are the most common siderophores in nature. These siderophores can be produced not only by bacteria but also by fungi. In bacteria, these hydrophilic siderophores are composed of acylated and hydroxylated alkylamines, such as the siderophore pyoverdine; produced by Pseudomonas fluorescens, while in fungi, they are composed of hydroxylated and alkylated ornithine [6], for example, trichoderma spp produces fecal coprogens. Except for the siderophore fusarinine C containing ester bond produced by Aspergillus fumigatus, other hydroxamate siderophores contain peptide chains [7]. A bidentate ligand is formed between the two oxygen molecules of the hydroxyformic acid group on the hydroxamic acid hydroxamate siderophores and iron. Each hydroxamate siderophores can form a hexadentate octahedral complex with a binding constant between 1022 L/mol and 1032 mol/L [8].

2.2. Catecholate Siderophores

Catecholate siderophores are a very well-studied class of siderophores that we are currently studying. They are found in bacteria, such as enterobactin produced by Escherichia coli and salmochelin secreted by Klebsiella pneumonia [9]. This type of siderophore is composed of catechol esters and hydroxyl groups, has dihydroxybenzoic acid (DHBA) coupled with amino acids, and adjacent hydroxyl or catechol ends can bind to Fe^{3+} [10]. This kind of siderophore has the characteristics of lipophilicity, high affinity to iron and strong resistance to environmental pH changes [11]. The catecholate siderophore group provides two oxygen atoms to chelate with iron to form a hexadentate octahedral complex, resulting in a binding constant of up to 1052 L/mol between enterobactin and iron.

2.3. Carboxylate Siderophores

Carboxylate siderophores are produced by a small number of bacteria, such as rhizobactin produced by Rhizobium meliloti. These siderophores are composed

of citric acid or β-hydroxyaspartic acid. For example, staphyloferrin A produced by Staphylococcus aureus contains one D-ornithine and two citric acid residues linked by two amide bonds. These siderophores can bind to iron atoms through carboxyl and hydroxyl groups. Under acidic pH conditions, carboxylate siderophore were able to chelate iron ions more efficiently than hydroxamate siderophores and catecholate siderophores.

3. Types of Siderophore Esterases

3.1. Bacterial Related Siderophore Esterases

Enterobactin (Ent) is a typical chelating agent, a cyclic tricyclic lactone composed of three dihydroxybenzoyl serine (DHB) monomers. In the cytoplasm, Ent is assembled through something called non-ribosomal peptide synthase. After that, it enters the periplasm through the membrane protein EntS and is finally transported to the environment through TolC. The iron binding (holo) Ent needs FepA to enter the periplasm and the subsequently move to the cytoplasm with the help of membrane proteins FepD and-G, and actuated by Fepc-mediated ATP hydrolysis [12]. Trilactone scaffold is completely hydrolyzed by esterase Fes and releases iron ions into the cytoplasm [13]. Although Ent has a high affinity for iron, its absorption efficiency is impaired *in vivo* due to at least one congenital defence mechanism in mammalian hosts. The host limits bacterial growth and virulence by utilizing epithelial cells and neutrophils to secrete the protein Lcn2 and allowing the protein Lcn2 to compete effectively with bacteria for binding ent with high specificity and affinity [14].

Lately, a c-glucosylated Ent analogs have been identified from salmonella strains, known as salmochelins [15] [16]. The production of Salmochelin may be related to pathogenic bacteria destroying the innate immunity of mammalian hosts. The catecholate arm of Ent is modified with the addition of a glucose group at the end to obtain salmochelin , which is structurally identical to it. Ent's glycosylation prevents L from binding to it, allowing uninhibited bacterial virulence and growth. So that we can conclude is that salmochelin is a better siderophore than Ent in the presence of Lcn2 [16]. Ferric Ent is sequestered by the protein siderocalin in a mammalian host [17], and glucosylation of Ent presumably decreases the affinity for siderocalin. The IroA gene cluster (IroBCDEN) plays an indispensable role in the synthesis, secretion, and utilization of salmochelins [18].

IroB has been proved to catalyze c-glycosylation of Ent, There is sufficient evidence to show that IroB forms C-C bonds by connecting a portion of glucose to the receptor molecule, catalyzing the generation of MGE, which is then converted into DGE, and finally produces a small portion of TGE. DGE (the same as salmochelin S4) is the main product generated by IroB catalysis. Just like salmochelin, through this form of molecular camouflage, it can prevent it from binding to Lcn2, thereby helping Ent evade the host's innate defense [19].

IroC is considered to be an intima transporter of the salmochelins, which

plays a role in the output of apo siderophores [20]. Apo salmochelins can be transported to the periplasm through IroC, while holo salmochelins can be input through receptor IroN. In the peripheral plasma, Fe^{3+}-bound salmochelins S4 was cut by IroC to form a linear trimer Fe^{3+}-bound salmochelins S2. IroC not only allows for the uptake of Fe^{3+}-salmonetin S2, but also allows for the uptake of Fe^{3+}-(DHBS) 3, which is the linear form of Fe^{3+}-enterocolin.

IroD is a Fe-MGE/Fe-DGE esterase, which tends to be in the ferric forms rather than apo forms as substrates. IroD has been shown to be a cytoplasmic esterase that can hydrolyze Fe-Ent, Fe-MGE and Fe-DGE into fragments with low affinity for ferric [13]. The ferric forms of these siderophores can be hydrolyzed into linear trimers, dimers and monomers by cytoplasmic esterase IroD. This is very similar to ent, where IroD degrades salmonelin S4 into linear trimer salmonelin S2, dimer salmonelin S1, monomer DHBS, and salmonelin SX. However, IroD is not only necessary for the release of iron in the form of iron, but also indispensable in the process of linearization and secretion after utilizing degraded siderophores. Therefore, in the absence of IroD, catechol siderophores cannot degrade and export to cells, thereby affecting the virulence and growth of bacteria.

Unlike IroD, IroE is periplasmic esterase and it tends to hydrolyze apo-MGE/DGE only once to produce these linear trimers. The catalytic efficiency (kcatKM-1) of IroE for hydrolysis of apolipoprotein was at least 16 times higher than that of Fe^{3+} binding form, and the hydrolysis of Fe^{3+} binding form siderophore was inhibited by the final substance in high concentration of substrate *in vitro* [13]. Secondly, the linear siderophore is more plentiful in the culture medium than the circular siderophore, while the circular siderophore is dominant in the IroE deletion strains. The highest DALI Z score 20 determines that esterase Fes has the highest structural homology with IroE [20]. The main topological structure difference between Fes and IroE is that Fes has a large amino-terminal domain covering a portion of the active site, which may be important for substrate recognition.

Fes is a cytoplasmic esterase that tends to be Fe-Ent rather than apo-Ent. The enzymology of Fes is similar to IroD, because both enzymes tend to use the holo siderophore as the substrate and completely cut the triolactone scaffold into DHB-Ser or Glc-DHB-Ser monomers. The efficiency of Fes catalyzing the hydrolysis of Fe-Ent is 20 times higher than that of Fe-MGE, and the catalytic efficiency of Fe-DGE or Fe-TGE is very small, almost without catalyzing the hydrolysis of them. Fe-ENT binds to the periplasmic binding protein FepB in the cytoplasm and is transported to the cytoplasm through the ABC transporter protein FepDGC [21] [22] [23] [24]. In the cytoplasm, the esterase Fes hydrolyzes ENT into three N-(2,3-dihydroxybenzoyl) serine (DHBS) molecules, which can still chelate iron [25] [26]. Fes protein can cleave Ent erobacter protein into linear DHBS trimer (DHBS) 3, dimer (DHBS) 2 and monomer DHBS. Fes has a large amino-terminal domain covering a portion of the active site. It is supposed that this structure may help Fes capture substrates and initial hydrolysis prod-

ucts, enabling Fes to further degrade siderophores and release iron. Thus, the hydrolysis reaction can proceed more successfully and degrade the trilactone scaffold completely.

PfeE, a kind of periplasmic esterase with high homology to iroe, can hydrolyze ferric-ent in P. aeruginosa into three molecules of 2,3-DHBS (2,3-dihydroxybenzoyl serine) which are still complexed with iron ion, and only in the presence of PfeE and iron reducing agents(such as DTT) can the Ent chelating group completely dissociate from iron ions. Ent is not only utilized by the microorganisms that produce it, but also as an exogenous siderophore by many bacteria that cannot synthesize it, such as Pseudomonas aeruginosa. What we already know is that in Pseudomonas aeruginosa, iron carrier hydrolysis only occurs in the cytoplasm, and Ent has never reached the bacterial cytoplasm [27].

Cee is also a type of periplasmic triterpenolase that can hydrolyze Fe-Ent in bacterial periplasmic material, and is the only Ent esterase in Campylobacter. Cee has the highest homology with IroE in terms of structure, however, whether apo-Ent or Fe-Ent, Cee has a higher hydrolysis rate and efficiency than IroE. By comparing enzyme activity measurements, it can be concluded that Cee is very similar in function to Fes and IroD. After passing through the outer membrane, Fe-Ent is hydrolyzed by periplasmic Cee to produce dimers and monomer products, causing immediate release/transfer and assimilation of iron in the periplasmic material [28].

3.2. Fungal Related Siderophore Esterases

Fungi are different from bacteria in that they mainly produce hydroxamate siderophore, and only a small amount of special fungi produce catecholate siderophore [29]. The hydroxamate siderophore of fungi can be divided into four categories: coprogens, ferrichromes, flusarinines and rhodotorulic acid. Their common feature is the formation of bidentate hydroxamic groups that can tightly bind with iron ions. Most fungal siderophores are connected by three ester or peptide bonds to form a hexagonal structure, in order to increase their affinity for iron ions. Moreover, the cyclization of iron carriers can also improve chemical stability. Aspergillus fumigatus can produce four types of low molecular weight iron chelating agents(called siderophores). This Includes two extracellular siderophores of Fusarium oxysporum type, Fusarium oxysporum alkaloid C (FsC) and its derivative acetyl Fusarium oxysporin C (TAFC), which are used to absorb iron. The other two intracellular siderophores of ferrichrome type, ferrite (FC) is used to distribute and store mycelium iron, and hydroxyferrite (HFC) is accumulated to distribute and store conidia iron [30] [31]. In terms of structure, FSC is composed of three N5 aminovalonyl-N5 hydroxide residues that are cyclically linked through ester bonds. TAFC is obtained by FSC N2 acetylation. FC is a cyclic hexapeptide with a structure of Gly Ser Gly-(N5-acetyl-N5-hydroxyornithine). HFC is obtained by hydroxylation of FC [32]. These siderophores have been proven to be crucial for the virulence of fungi [33]. After iron chelation and uptake, FSC and TAFC are hydrolyzed, and iron is trans-

ferred to the metabolic or intracellular siderophore FC for transportation and storage [34] [35].

After uptake, TAFC and FSC iron complexes need to be hydrolyzed in the cytoplasm using siderophore esterases. Two types of siderophore esterases have been discovered, namely esterases EstB and SidJ, which come from two different protein families.

EstB is a TAFC esterase located in the cytoplasm. Compared with wild strains, EstB deficient strains have the same growth rate under iron rich conditions, but the growth rate of deficient strains is significantly slowed down under iron deficiency and the presence of BPS. We know that BPS is a ferrous specific chelating agent that can inhibit reducing iron assimilation, so in this case, the siderophore system becomes the only functional iron absorption system in Aspergillus fumigatus. When EstB is lacking, TafC accumulates in cells due to reduced hydrolysis, which also reduces the transfer of TAFC like intracellular siderophore FC, leading to delayed iron sensing and ultimately affecting fungal growth and virulence [36].

SidJ is an FSC esterase with high specificity for it. Like EstB, the growth of SidJ deficient plants was significantly slowed down compared to wt under iron deficiency and the presence of BPS. The lack of SidJ increases the accumulation of FSC and its degradation products, and reduces the transfer rate of iron to FC. This delays the iron induction. Lack of FC， vacuole storage, and especially lack of both can increase the cellular content of iron chelated by siderophore decomposition products, indicating that iron is transferred from extracellular siderophores to metabolism, and FC or vacuoles are recovered before siderophore decomposition products [37].

4. Discussion

Iron is essential for the survival of almost all living things. Iron is not only an important component in the synthesis of hemoglobin and myoglobin, but is also involved in the transport and storage of oxygen. It is also essential for biological DNA synthesis, energy production, and cellular respiration [38]. However, because of its redox capacity, iron can promote the formation of hydroxyl or lipid radicals, which can damage proteins, DNA, and lipids. Therefore, biological maintenance of iron homeostasis requires a variety of complex pathways [39] [40]. One of the most common pathways is through the secretion of siderophores, which works primarily in the presence of iron deficiency. Bacteria primarily secrete catecholate siderophores, such as ENT, and Salmochelin. Fungi mainly secrete hydroxamate siderophores, which are mainly categorized as coprogens, ferrichromes, flusarinines, and rhodotorulic acid. In the process of siderophore transportation of iron ions, the help of siderophore esterase is indispensable. Siderophore esterase mainly through the iron ions bound to the siderophore hydrolyzed into more small molecules, so as to achieve the purpose of cellular iron uptake.

The problem of antimicrobial resistance, whether in bacterial or fungal infections, has become so serious that it poses a great threat to the lives and health of people around the world, and a new strategy is urgently needed to solve the problem of antibiotic resistance, such as finding a new antibiotic target or modifying and improving the existing antibiotics in terms of the resistance mechanism. Currently there is a strategy to promote antibiotic uptake by using siderophores to mediate bacterial translocation, called the Trojan horse delivery strategy [41] [42] [43]. The research has been somewhat successful and many siderophore drug conjugates have entered clinical trials. It involves narrowing the spectrum of antibiotic activity by covalently linking the siderophore to the antibiotic, targeting growth inhibition of the pathogen by transporting the antibiotic molecules together as the siderophore transports iron ions to the target bacteria [44]. However, antibiotics become inactive when bound to siderophores and need to be hydrolyzed by siderophore esterases in order to regain activity. IroD esterases have been shown to restore the activity of ciprofloxacin by hydrolyzing ciprofloxacin conjugated to Ent [45]. Some of the previously mentioned siderophore esterases may also be a good research direction for the Trojan horse strategy.

In conclusion, this paper provides a structural and functional overview of several of the most common siderophores as well as siderophore esterases, and we already know that iron has an irreplaceable role in the growth and virulence of bacteria and fungi, and that the siderophore system is also a very important part of biological iron metabolism. An in-depth understanding of the process of iron uptake by organisms through the use of siderophores and siderophore esterases can make an important contribution to the development of new antibiotics or the improvement and modification of existing antibiotics.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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