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Review

Synthesis, nature and utility of universal iron chelator – Siderophore: A review

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ABSTRACT

Siderophores, the secondary metabolite of various microorganisms are ferric ion specific chelators secreted under iron stressed condition. These non-ribosomal peptides have been classified as catecholate, hydroxamate, carboxylate and mixed types. Recent studies focus on discovery of possible mammalian siderophores. The biosynthesis pathway including non-ribosomal dependent as well as non-ribosomal independent pathways are of great interest now a days. Many significant roles of siderophores such as virulence in pathogens, oxidative stress tolerance, classification of organisms etc. are being discovered. Studies on siderophore utilization in bioremediation and other heavy metal chelation have increased in past decade. The iron chelation ability of siderophores is being recently studied with regards to malignant cancerous cells. Not only this, it has been found that they possess antimicrobial properties which can be utilized against number of microbes. This review covers all recent aspects of siderophore and its applications.

1. Introduction

Iron is essential for most of the growth and developmental processes of every living organism. In natural habitat the ferric iron has a solubility of 10–17 M at neutral pH but certain microbes like bacteria require 10-5-10–7 M of iron for their optimal growth. Also in human serum, the iron transport protein transferrin maintains ferric iron concentration at around 10–24 M which makes it inaccessible for the pathogens (Raymond et al., 2003). In earth's crust, even after being the fourth most abundant element, ion is not easily accessible to these microorganisms. This is because iron at biological pH and aerobic conditions gets oxidized to insoluble oxyhydroxide polymers (Paul and Dubey, 2015). Microbes have evolved number of ways for iron scavenging. One of the ways includes siderophore-mediated acquisition of iron through specific receptor and transport system. Siderophores (Gk.sidero-iron, phores-carrier) are high affinity ferric ion specific chelator with low molecular weight of less than 10 KDa, excreted under iron starvation by various micro-organisms like bacteria, fungi and also by some plants. Some mammalian siderophores have also been reported recently (Devireddy et al., 2010). At least one type of siderophore has been reported in most aerobic and facultative micro-organisms (Neilands, 1995). Siderophore not only aids in iron acquisition from insoluble hydroxide forms but is also involved in its acquisition from ferric citrate, ferric phosphate, ferric transferrin, iron bound to plant flavone pigment, sugars and glycosides (Winkelmann, 2002). In general, the acquisition of iron starts with binding of excreted siderophores with available ferric ion forming a ferri-siderophore complex and then binding of this complex to specific receptor protein present on microbial cell surface. The complex gets translocated by active transport and is released inside the cell. Siderophores coordinate to ferric ions through lewis base having hard donor atoms, such as negatively charged oxygen or neutral nitrogen and oxygen, as iron is a hard lewis acid (Boda et al., 2016). Production of siderophores decreases with an increase in iron concentration in the surroundings (Singh et al., 2008). Reports suggest strong influence of iron (III) concentration on siderophore production by Gram − positive bacteria namely *Micrococcus luteus* and *Bacillus silvestris* (Cabaj and Kosakowska, 2009).

Microorganisms such as *Escherichia coli* not only utilize their own siderophores but can also use siderophores secreted by others like fungi. Siderophores are also involved in ligand exchange reaction as reported in case of *Aeromonas hydrophila* in which there is an exchange of iron from a ferric siderophore to an iron-free siderophore bound to the receptor. This ligand exchange

mechanism mediated by siderophore occurs at cell surface (Stintzi et al., 2000). For acquisition of iron from its surrounding micro-organisms employ different strategies including the siderophore mediated iron uptake. Bacteria solubilize ferric oxides to meet iron requirement either by lowering external pH or by reducing ferric iron to the relatively soluble ferrous form. Another strategy is to utilize iron chelators like siderophores (Fig. 5). Siderophore mediated iron uptake requires specific outer membrane (OM) receptors like FepA, FecA and FhuA, which bind to their cognate ferrisiderophore complex (Köster, 2001).

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All the receptors share same structural property and consist of a 22 antiparallel β-stranded β- barrel that contains extracellular loops which interact with the ferric-siderophore complex and a hatch domain at N-terminus (Krewulak and Vogel, 2008). Transport of the ferrisiderophore complex across periplasm and cytoplasmic membrane requires certain binding protein that acts as a shuttle. These binding proteins like FhuD, collect the ferri-siderophore released from outer membrane receptor and deliver it to a cognate permease like ABC permease present in the inner membrane. In *E. coli,* when ferric siderophore complex gets internalized the ester bond between the complex is hydrolyzed by esterase protein encoded by *fes* gene, resulting in release of iron and producing dihydroxybenzoyl serine that acts as a weak siderophore (Stintzi et al., 2000; Köster, 2001).

Aspergillus fumigatus is reported to acquire iron by reductive iron assimilation (RIA) and ferrous uptake besides the siderophore mediated pathway (Schrettl et al., 2007). *A. fumigatus* exploits the RIA pathway during lack of extracellular and intracellular siderophore in vitro. In reductive iron assimilation pathway, ferric form of iron gets reduced to ferrous form and is taken up by an FtrA/FetC complex. When RIA is ineffective the extracellular siderophore promotes hyphal growth under iron limitation. In *A. fumigatus*, *ftrA*, a high affinity iron permease encoding gene, when deleted results in increased production of siderophore to compensate for the lack of RIA (Schrettl et al., 2004). However, *Aspergillus nidulans* lacks the capacity for reductive iron assimilation.

In *Pseudomonas*, iron acquisition is carried out through pathway involving FpvR, FpvA and PvdS protein (Lamont et al., 2002). Upon formation of a ferri-pyoverdine complex it interacts with the cell surface receptor i.e. FpvA, part of which in turn interacts with FpvR located on periplasm. This initiates a signaling cascade inducing PvdS that activates RNA polymerase. This initiates transcription of pyoverdine which again binds to ferric iron. The *pvdS* gene activity is controlled by Fur, an iron-sensing repressor protein which allows *pvdS* transcription only under iron starved condition (Cunliffe et al., 1995).

2. Types of siderophore based on chemical properties and structure

Siderophores are known to differ from one another in their chemical structure and properties. Based on their chemical nature, siderophores have been classified into hydroxamate, catecholate and carboxylate types. Besides the above mentioned types certain siderophores are categorized as mixed type (Fig. 1).

2.1. Hydroxamate siderophore

In bacteria, these hydrophilic siderophores are made up of acylated and hydroxylated alkylamines while in fungi these are hydroxylated and alkylated ornithine based (Baakza et al., 2004). It consists of N5-acyl-N5-Hydroxyornithine or N6-acyl-N6-Hydroxylysine (Winkelmann, 2002). Except fusarinine C produced by *Aspergillus nidulans* (e.g.; TAFC) which contain ester bonds, all other hydroxamate contain peptide linkage (Oberegger et al., 2001). A bidentate ligand forms between two oxygen molecules coming from each hydroxamate group and iron. Each hydroxamate is capable of forming a hexadentate octahedral complex with ferric ion with a binding constant in the range of 1022–1032 M^{-1}(Saha et al., 2016).

2.2. Catecholate siderophore

This type of siderophore is found only in bacteria. It consists of catecholate and hydroxyl groups and binds Fe3+ with adjacent hydroxyls or catechol ends (Paul and Dubey, 2015). It has dihydroxybenzoic acid (DHBA) coupled to an amino acid. Lipophilicity, complex stability and resistance to environmental pH are its unique properties (Winkelmann, 2002). Catecholate siderophore group also forms hexadentate octahedral complex by providing two oxygen atoms for iron chelation (Saha et al., 2016).

2.3. Carboxylate siderophore

Produced by few bacteria, such as rhizobactin produced by *Rhizobium meliloti* and fungi, like members of mucorales belonging to zygomycota, these siderophores have carboxyl and hydroxyl group for iron acquisition. It consists of citric acid or β-hydroxyaspartic acid that binds with iron such as in staphyloferrin A, excreted by *Staphylococcus aureus* that consists of one D- ornithine and two citric acid residues linked by two amide bond. Some organisms produce mixed siderophores containing both catecholate as well as hydroxamate group, such as heterobactin produced by *Rhosococcus erythropolis* (Paul and Dubey, 2015).

3. Types of siderophore based on producing organism

3.1. Fungal siderophore

Generally, fungi produce hydroxamate and carboxylate type of siderophores. Widely studied fungi for siderophore production are *Aspergillus fumigatus* and *Aspergillus nidulans* having 55 similar types of siderophores. Both *A. fumigatus* and *A. nidulans* are saprophytic fungi playing an essential role in recycling of environmental carbon and nitrogen. *A. fumigatus* is responsible for causing aspergillosis in immunocompromised patients whereas not so hazardous *A. nidulans* is widely used as model organism in research. Hydroxamate siderophore fusarinine C and triacetyl fusarinine C (TAFC) are utilized by *A. fumigatus* for capturing extracellular iron, hyphal siderophore ferricrocin for intracellular iron distribution and storage and conidial siderophore hydroxyferricrocin for conidial iron storage, germination and oxidative stress resistance (Schrettl et al., 2010b). Ferricrocin and ferrihordin are the main siderophores excreted by *A. nidulans*. It has been observed that a 24 h old culture of *A. nidulans* produces fusigen, an unacetylated form of TAFC while in older culture (~48 h) TAFC production is observed as a result of breakdown or uptake of fusigen (Oberegger et al., 2001).

In two ectomycorrhizal basidiomycetes, *Laccaria laccata* which is common woodland fungus and *L. bicolor*, a model organism used in research, the principal siderophore reported is the ester containing siderophore linear fusigen besides coprogen, ferricrocin and triacetylfusarinine C in small quantities (Haselwandter et al., 2013). Other than hydroxamate, the brown-rot fungus Wolfiporia *cocos,* a basidiomycota member used in Chinese medicine, has also been reported to produce catecholate siderophore (Arantes and Milagres, 2008).

3.2. Bacterial siderophore

Bacteria produce only extracellular forms of siderophore. Most widely studied bacteria for siderophore production is Gram − negative, facultative anaerobe *Escherichia coli,* normally found in intestine. *E. coli* principally produces enterobactin, a catechol siderophore with highest affinity towards Fe (III) ion than any other known siderophore. It is a triscatechol derivative of cyclic triserine lactone (Raymond et al., 2003). Diverse types of siderophore have been reported in Gram-positive actinobacteria. For example the spore producing, Gram − positive member of family Streptomycacetaceae − *Streptomyces* produces desferrioxamine siderophores, such as desferrioxamine G, B, and E and tuberculosis causing actinobacteria *Mycobacterium* produces extracellular siderophores, carboxymycobactins and exochelins (Winkelmann 2002). The pathogenic Gram − negative coccobacillus *Yersinia pestis* the causative agent of deadly plague is characterized by a high pathogenic island that encodes yersiniabactin (Ybt) siderophore (Paauw et al., 2009). Ybt is able to remove iron directly from transferrin and use iron from lactoferrin Fetherston et al., 2010). The strain 6A of Gram-negative, nosocomial, multi drug resistant, aerobic