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REVIEW ARTICLE

The Review for Nonfluorescent Siderophores Produced by Pseudomonads

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Abstract

Siderophores are small, high-affinity iron-chelating compounds that are secreted by microorganisms such as bacteria and fungi and serve primarily to transport iron across cell membranes. Iron is an indispensable trace element in virtually all living organisms. Under iron-limiting conditions bacteria and fungi produce siderophores to assimilate iron. Due to their highly elaborate iron-uptake systems, Pseudomonads are able to colonize in many iron-restricted environments. Pseudomonads can secret two kinds of siderophores:fluorescent siderophores and nonfluorescent siderophores. There are many kinds of nonfluorescent siderophores produced by Pseudomonads, and their functions are various. Recent studies show that nonfluorescent siderophores have important clinical and environmental application value. In this review, the main types and additional functions of nonfluorescent siderophores produced by Pseudomonads are discussed.

Keywords: Siderophores; Pseudomonads; Nonfluorescent Siderophores; Function

Introduction

Siderophores are high-affinity iron chelators that can combine Fe(III) from the environment and transport it into cells effectively via membrane-associated transport systems [1,2]. Iron is crucial for almost all organisms to maintain normal life, as it acts as a cofactor for many metabolic enzymes mediating redox reactions and electron transfer [1,3]. However, the iron available in the environment is insufficient because of its low solubility under physiological pH and aerobic conditions, which has led to the development of elaborate high-affinity iron uptake systems in many organisms [4]. However, excess free iron can be deleterious due to the formation of oxygen radicals [5]. Consequently, iron uptake must be tuned carefully to avoid both starvation and toxicity [6]. Microorganisms can synthesize and secrete siderophores to overcome iron deficiency [4,7,8]. Many bacteria produce more than one siderophore: one primary high-affinity siderophore and one or several lower-affinity siderophores. By employing and regulating these siderophores, bacteria can adjust to changing iron conditions in the environment and maintain intracellular iron homeostasis.

Pseudomonads are widely distributed in diverse niches and are extremely versatile and adaptable. This adaptability is thought to be associated with their diverse and hierarchical siderophore systems [9,10]. Among the various siderophores of pseudomonads, the fluorescent high-affinity peptidic pyoverdines are usually produced as the fluorescent siderophore and have been studied intensively [10-12]. Diverse nonfluorescent siderophores are less well understood because their production and functions are usually masked by pyoverdines. Recently, it has been found that these nonfluorescent siderophores have many other functions, such as the decomposition of drugs, carcinogens, organic compounds, antibiotic function, oxidant, inducing the formation of plant defense mechanism, and acting as a catalyst in the process of biodegradation. Microorganisms may use the antibacterial or other activities of these nonfluorescent siderophores to improve the competition of these bacteria.

Therefore, the study of these nonfluorescent siderophores produced by Pseudomonads will help us to further understand the evolution of bacteria such as Pseudomonads and their diversity of survival strategies in response to the environment, which is beneficial for the exploration and application of nonfluorescent siderophores.

The main types of nonfluorescent siderophores

(Thio) Quinolobactin

Quinolobactin is secreted by *Pseudomonas fluorescens* ATCC 17400 under conditions of iron limitation(13), which itself results from the hydrolysis of the unstable molecule 8-hydroxy-4-methoxy-2-quinoline thiocarboxylic acid(thioquinolobactin)(14). The molecular mass of quinolobactin is 219 Da. The structure of quinolobactin is shown in Fig.1. Quinolobactin can form a complex with Fe(III). When free quinolobactin is examined, two absorbance peaks are observed at 216 and 249 nm; when the iron complex is examined, peaks are observed at 212 and 250 nm. Fe (III) is coordinated through the nitrogen and the oxygen quinoline atoms. Quinolobactin forms complex of the 2:1 stoichiometry(two ligands for one iron). There is no evidence of coordination by the carboxylate group. Presumably, the carboxylate coordination could be unfavored to achieve the octahedral environment around Fe (III), but remains close to the coordination sphere.

The structure of thioquinolobactin is similar to that of quinolobactin (Figure 1). Thioquinolobactin is unstable and easy to be hydrolyzed into quinolobactin. The molecular mass of thioquinolobactin is 235 Da (15).

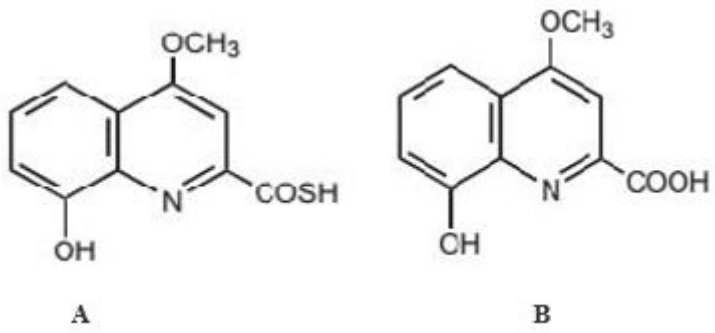

Figure 1: Structure of Thioquinolobactin (A) and Quinolobactin (B)

Corrugatin is kind of lipopeptides, which is secreted from *Pseudomonas corrugata.* Corrugatin has several structural pecularities, the most notable being the rarely encountered amino acid ß-OHHis [16]. The peptide part of corrugatin contains D-and L-Ser in equal amounts, Dab(at least one with a free ϒ-NH2-group), L-threo-ß-OHHis, and C-terminal L-thre-ß-OH-Asp with free COOH-groups. The stoichiometry of corrugatin ferric complex is determined to be 1:1. The corrugatin ferric complex is yellow. At pH 7.4, the UV/Vis spectrum of corrugatin shows a strong absorption band at 260 nm and weak ones at 375 and 450 nm. The low intensity of the bands at 375 and 450 nm suggests that they are due to charge-transfer transitions.

Ornicorrugatin was obtained from *Pseudomonas fluorescens* AF76, which is also a lipopeptidic siderophore. Comparison of corrugatin and ornicorrugatin, they have identical composition with the exception that ornicorrugatin contained an additional D-Orn unit (Figure 2) [17].

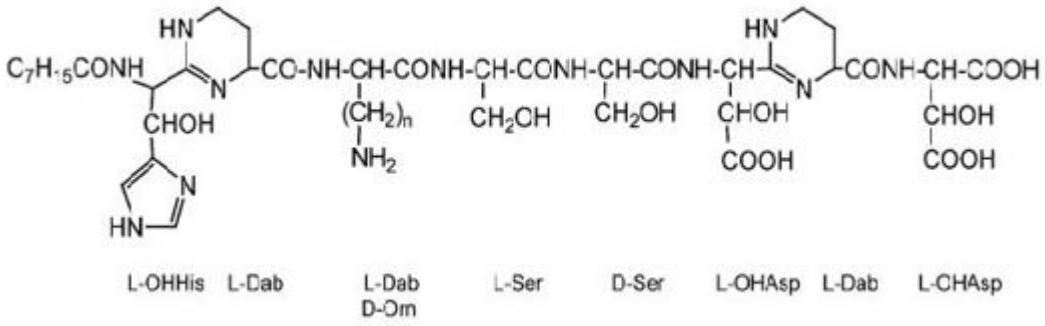

Figure 2: Stricture of Corrugatin (1, n = 2, Dab) and Ornicorrugatin (2, n = 3, Orn)

Pyridine-2,6-dithiocarboxylic acid

Pyridine-2,6-dithiocarboxylic acid(PDTC) is a new nonfluorescent siderophore secreted by certain *pseudomonas* when grown aerobically or anaerobically in iron-limited laboratory media, which was originally used as an active material to degrade carbon tetrachloride [18]. The molecular mass of PDTC is 198 Da. The structure of PDTC is shown in Figure 3. The stoichiometry of PDTC ferric complex is determined to be 2:1. At pH 2, the UV/Vis spectrum of PDTC shows a strong absorption band at 270 nm and 335 nm. PDTC and thioquinolobactin belong to thiocarboxylate, which may be a new classification of nonfluorescent siderophores different from oximate and catechol [19].

One of the unique properties of PDTC is its high affinity but low specificity for transition metal ions including Cu(II), Co(III), Fe(III), and Ni(II). PDTC's unique structure contributes to its relative nonspecificity for ligands. Sulfur and nitrogen, both soft bases, are more specific for metals with soft character. So, by virtue of its binding atoms, PDTC has a natural affinity for soft, easily polarizable metals like, Au(I), Cd(II), Cu(I),Hg(I), Hg(II), and Pd(II). However, the proximity and orientation of PDTC's two sul-

fur and single nitrogen binding atoms, which essentially form a tridentate'binding pocket', evidently increases the binding affinity of this molecule for hard metals like Bi(III), Co(II),Co(III), Cr(III), Cu(II), Fe(II), Fe(III), Ni(II), Pb(II) and Zn(II) over that which would be expected for independent S and N binding atoms(20).

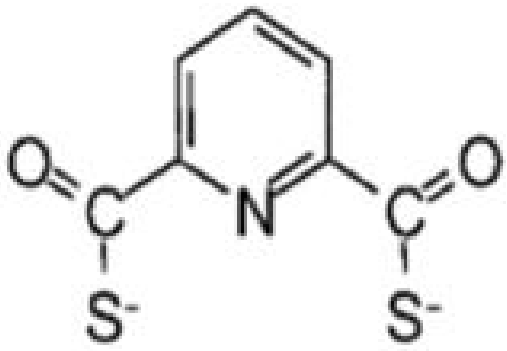

Figure 3: Structure of PDC

Tropolone

In 1945, the term tropolone was initially proposed by Dewar to describe a seven-membered ring structure. Tropolone, 2-hydroxy-2,4,6-cycloheptatriene-l-one, has a molecular weight of 122 Da and forms colorless and transparent needle, prismatic, and tabular crystals. It is a nonbenzenoid aromatic compound and has properties characteristic of phenols and acids. Various natural tropolones products have been discovered in plants, fungi, and bacteria [21,22]. In bacteria, tropolone has been found to be produced by *Pseudomonas ATCC 31099* and *Pseudomonas plantarii* ATCC 43733 [23,24]. Tropolone forms a red complex with Fe(III) at a ratio of 3:1 [24].

Pseudomonas donghuensis can excrete large quantities of iron chelating substances in ironrestricted environments(25). At least two kinds of ironchelator can be found in the culture supernatant: fluorescent siderophore pyoverdins, and an ethyl acetate-extractable nonfluorescent siderophore [26]. The nonfluorescent siderophore was the dominant contributor to the iron chelating activity of the culture supernatant of *Pseudomonas donghuensis*. Electron ionization mass spectrometry, NMR spectroscopy, and IR spectroscopy identified the nonfluorescent siderophore as 7-hydroxytropolone with the mass of 138Da, which is the derivative of tropolone. The stoichiometry of 7-hydroxytropolone ferric complex is 2:1. At pH 6.7, the UV/Vis spectrum of 7-hydroxytropolone shows a strong absorption band at 327 nm and 394 nm. 7-Hydroxytropolone can form complex with Fe(III) in a stoichiometry of 2:1, which is different from tropolone [24]. The ligands should be the carbonyl and hydroxyl oxygen atoms. This is the first time that 7-hydroxytropolone has been shown to act as a siderophore for its producer.

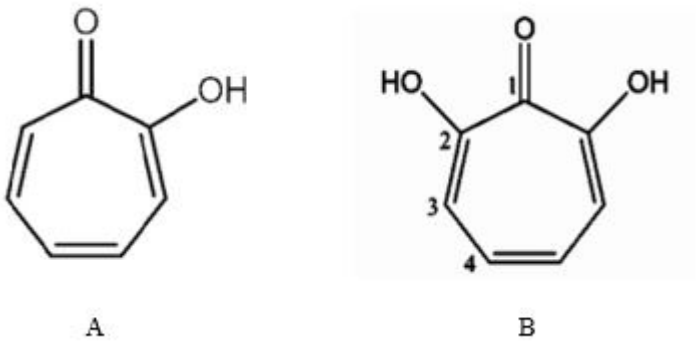

Figure 4: Structure of tropolone (A) and 7-hydroxytropolone (B)

Additional functinons of nonfluorescent siderophores

Further illustrating the roles of the two siderophores' it indicates that nonfluorescent siderophores tends to be produced under less severely iron-depleted conditions, while fluorescent siderophore is produced under extremely iron-depleted conditions [26]. The coexistence and complementation of the two different siderophores may enable bacteria to adapt to a wider and more variable external iron environment. In addition, the expression of fluorent siderophore systems is believed to require a set of synthesis, assembly, and transport machineries to be triggered and thus consumes a lot of energy [27]. The fact that Pseudomonads produces different siderophores under different iron conditions indicates that Pseudomonads may produce the siderophore that is most suitable for the iron status and thereby avoid wasting energy [26].

Diverse nonfluorescent siderophores are less well understood because their production and functions are usually masked by fluorescent siderophore' pyoverdine. However, these nonfluorescent siderophores could play essential roles in the growth of pyoverdine-negative mutants under iron-limited conditions and could provide additional functions, such as pathogenicity,biocontrol, and transport of other metals [28-30].The potential application value from additional functions of these secondary siderophores far exceeds its function of iron scavenging.

Antibacterial Effect

The nonfluorescent siderophore thioquinolobactin, TQB, has antibacterial effect and *in vitro* anti-*Pythium* activity of *Pseudomonas fluorescens* ATCC 17400 is for the major part due to the nonfluorescent siderophore thioquinolobactin, TQB [14]. In 1975, it was reported that the nonfluorescent siderophore tropolone was a broad-spectrum antibacterial agent, which had antibacterial effect on Gram-positive and Gram-negative bacteria. When the concentration of tropolone was more than 0.02 mol / L, it had bactericidal effect on both Gram-positive and Gram-negative bacteria [31]. The nonfluorescent siderophore pyridine-2,6-dithiocarboxylic acid, PDC, produced by *Pseudomonas stutzeri* and *Pseudomonas putida*, also has antibacterial effect, and its antibacterial effect needs metal as the co-factor. Similarly, pyochelin (PCH), the nonfluorescent siderophore of *Pseudomonas aeruginosa*, forms a complex with vanadium which is toxic for the cells [32]. It is possible that microorganisms can enhance the competitiveness and inhibit the growth of pathogens by secreting these antibacterial the nonfluorescent siderophores.

The concept of biological disease control, particularly using nonpathogenic bacterial strains for disease prevention, has received widespread attention during the last decade. When tested in vitro, iron limitation has been found to facilitate the antibacterial effect of fluorescent pseudomonads [33,34]. Thus, inhibition may be due to the production of these nonfluorescent siderophores, which deprive the pathogen of iron. Therefore, these nonfluorescent siderophores can be used as a potential antibacterial agent.

Effect of bioremediation

Metal plays an important role in the development of human civilization, but manufacturing, sludge application, nuclear power plant and mining all lead to metal pollution. The nonfluorescent siderophores are very effective in dissolving and improving the mobility of most metals. For example, the nonfluorescent siderophore pyridine-2,6-dithiocarboxylic acid was originally used as an active material to degrade carbon tetrachloride, PDC, which can form complex with various metals. This ability of the nonfluorescent siderophores depends on their ligand function, which indicates that they may have high affinity or selectivity for specific metals. Therefore, it is a low-cost, high-efficiency and environment-friendly bioremediation technology to use the nonfluorescent siderophores as bioremediation active agent.

Therapeutic effect on iron overload

Bacteria can regulate iron uptake by their highly elaborate iron-uptake systems to maintain normal growth and metabolism. Iron uptake and excretion in normal human body also maintain a dynamic balance. However, the accumulation of excessive iron has toxic effects on many organs. These iron overload can also lead to iron metabolism disorders, such as severe thalassemia and intermediate thalassemia, which can result in increased morbidity and mortality.

In order to prevent iron poisoning, too much plasma iron must be removed. Iron chelation therapy is to use nonfluorescent siderophores as medicine, which can reduce and eliminate the morbidity and mortality related to iron poisoning in many cases. For example current drugs in clinical use for the treatment of iron overload are the natural nonfluorescent siderophore desferrioxamine B. Desferrioxamine B was approved for the clinic by the US Food and Drug Administration (FDA) in 1968. Desferrioxamine B is a hexadentate hydroxamate siderophore, binding Fe(III) tightly in a stoichiometry of 1:1[35]. It has a good therapeutic effect on iron overload.

Effect of antibiotic carrier

Pathogenic microbes rapidly develop resistance to antibiotics. To keep ahead in the "microbial war", extensive interdisciplinary research is needed. A primary cause of drug resistance is the overuse of antibiotics that can result in alteration of microbial permeability, alteration of drug target binding sites, induction of enzymes that destroy antibiotics(ie., beta-lactamase) and even induction of efflux mechanisms. A combination of chemical syntheses, microbiological and biochemical studies demonstrate that the known critical dependence of iron assimilation by microbes for growth and virulence can be exploited for the development of new approaches to antibiotic therapy.

At present, an effective way to solve bacterial drug resistance is to connect antibiotic molecules with siderophores and transport them to bacterial cells [36]. For example, ferromycin can spontaneously covalently bind with siderophores, these siderophores-antibiotic complexes depend on the iron uptake system mediated by siderophores' which can be transported to the cells through the extracellular membrane and cytoplasmic membrane, and the drugs will be released through the intramolecular circulation during the iron reduction process. This is beneficial to increase the absorption of antibiotics, bypass bacterial resistance, improve the utilization rate of antibiotics, and stimulate the synthesis design of new antibiotics based on siderophores. so as to overcome the problem of bacterial resistance in clinical and improve the anti infection treatment.

Effect of metalloproteinase inhibitors.

Almost half of the proteases in organisms are metalloproteinases, which are involved in many different biological reactions. Their disorders are often related to cancer, inflammation, hypertension, bacterial and viral infections and other diseases. The metals in metalloproteinases, as auxiliary factors, generally play a basic catalytic role. Therefore, metal binding groups are common structures in metalloproteinase inhibitors.

Early studies have found that the nonfluorescent siderophores produced by microorganisms, such as iron desensitization, iron pigment and red yeast acid have a concentration dependent inhibition on zinc-containing matrix metalloproteinases(MMP-2) [37]. Later, it was found that the nonfluorescent siderophores can inhibit human 5-lipoxygenase, and the active site of 5-lipoxygenase contains non heme iron atom, which plays a key role in the biosynthesis of leukotriene. Leukotriene, as a medium of inflammatory reaction, is known to promote the pathogenesis of myeloid leukemia, so nonfluorescent siderophore has anti leukemia effect [38]. In addition to 5- lipoxygenase, the nonfluorescent siderophore also has an inhibitory effect on the Ribonucleotide reductas that play a role in carcinogenesis [39].

Due to the diversity of molecular structure of the nonfluorescent siderophores and their different degree of specificity with Fe (III), these natural products are expected to be candidates for the identification and design of new drugs targeting metalloproteinases.

Outlook

The nonfluorescent siderophores produced by Pseudomonads are not only great significant to microbial life activities, but also used in clinical disease treatment and environmental improvement. Therefore, it is great significant to study nonfluorescent siderophores produced by Pseudomonads.

Combining macrogenomics with detailed chemical analysis, we have made great efforts to study the variability, structure and functional characteristics of nonfluorescent siderophores, focused on finding effective methods, and applied nonfluorescent siderophores in the fields of bioremediation, biological control and medical treatment. These studies will contribute to the development of new applications of nonfluorescent siderophores.

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