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Microbial Ligand Coordination: Consideration of Biological Significance

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Abstract

Siderophores are generally considered to be microbial chelating compounds secreted by bacteria to facilitate uptake of iron(III). Certain siderophores, however, have a high affinity for other transition metal ions, including manganese(III), copper(II), molybdenum(VI), and vanadium(V). A new class of microbial ligands produced by methanotrophs, called chalkophores, is produced to facilitate copper uptake. This review considers the coordination of siderophores to Mn(III), Cu(II/I), Mo(VI) and V(V), and their stability constants relative to Fe(III), when available, as well as the coordination complexes of chalkophores to Cu(I).

Keywords: Siderophore, Iron, Manganese, Copper, Molybdenum, Vanadium

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1. Introduction

Bacteria growing aerobically in a low iron environment, have evolved an iron(III) uptake strategy based on the secretion of chelating ligands that sequester and solubilize iron(III) from environmental iron oxides or directly from iron proteins in a host organism. These microbial ligands, termed siderophores, reflect the Greek root, *sideros*, for iron, and *phore*, for carrier. Along with siderophores, the producing bacterium expresses an outer membrane receptor protein that is specific for a particular siderophore and catalyzes the transport across the outer membrane. ABC-type transport proteins in Gram-negative bacteria direct the iron siderophore

complex through the periplasmic space and across the cytoplasmic membrane [1-4]. While Gram-positive bacteria lack an outer membrane and periplasm, the process of iron-siderophore import is still catalyzed by ABC-type transport proteins. Relatively recently a new ligand exchange mechanism of Fe(III)-siderophore transport has been reported for the Gram-negative *Aeromonas hydrophila* [5] and the Gram-positive *Bacillus cereus* [6].

Many siderophore structures have been isolated and characterized from both Gram-negative and Gram-positive bacteria [7]. The common bidentate ligands within siderophores, include catechols, hydroxamic acids, and α -hydroxy carboxylic acids, but coordination by other groups is well established also, including carboxylic acids, oxazolone, and thiazolidine groups, among others.

The stability of an iron(III)-siderophore complex is reflected in the significantly negative reduction potential, which in turn is consistent with the selectivity of siderophores for Fe(III) over Fe(II). The relationship between the reduction potential and the stability of the iron(III) complex is described by a modified Nernst equation (Equation 1), where E°_{aq} is the reduction potential of free aqueous $\text{Fe}^{3+}_{(\text{aq})}$ [8]

$$E_{\text{Sid-FeIII/II}} = E^\circ_{\text{aq}} - 59.15 \text{Log}(K_f^{\text{FeIII}}/K_f^{\text{FeII}}) \quad (1)$$

Thus, hexacoordinate Fe(III) complexation by most siderophores, and negative reduction potentials of these Fe(III)-siderophore complexes mitigates against Fenton reactions and effectively controls the redox chemistry of iron(III) during uptake and transport [8].

Coordination chemists have been interested in the complexation of transition metal ions other than Fe(III) to siderophores since the initial discovery of siderophores [9-12]. Ga(III) and Al(III) are often used in place of Fe(III) to investigate complexation modes of siderophores by NMR. Lanthanide and actinide coordination to siderophores is established [13-15], although

possibly not of direct biological relevance. Chromium(III) complexes of siderophores are often prepared because the d^3 electronic configuration dictates slow ligand substitution in an octahedral complex [16] and thus these complexes are useful to determine the fate of a metal-siderophore complex in the initial stages of microbial uptake, however Cr(III)-siderophores are not biologically relevant, and in fact toxic to bacteria [16]. Following the initial discovery of siderophores, many investigations with divalent transition metal ions have been carried out [17, 18], although these ions are generally displaced by Fe(III).

The focus of this review is recent investigations on complexation and transport of manganese, copper, molybdenum, and vanadium by siderophores and also chalkophores, which is a new microbial ligand class produced by methanogens and is specific for Cu(I). Along with iron and copper, manganese is an important micronutrient which many organisms use as a redox cofactor [19, 20]. Bacteria have a variety of biological routes to obtain manganese, yet like iron, manganese is often found in the environment in oxide minerals composed of Mn(III) or Mn(IV) [21]; thus siderophore-mediated transport of Mn(III) could appear to be functionally similar to siderophore-mediated Fe(III) transport. Bacteria that fix dinitrogen have a high demand for iron, but most also a requirement for molybdenum to be incorporated in nitrogenase, or vanadium to be incorporated in the alternative nitrogenase [22]. A previous report suggested that siderophores could facilitate the uptake of molybdate into microbial cells[23], although without the use of a radioactive isotope (e.g., ^{99}Mo) or isotope ratio mass spectrometry (which was carried out later; see below), or even investigating transport- deficient mutants, siderophore-mediated uptake of metals other than Fe(III), had not been conclusively established, until recently.

2. Manganese Complexation by Siderophores

Manganese is abundant in surface waters of the Black Sea, where it is found at μM levels as Mn(III)L and fully complexed by a ligand or class of ligands, “L” [24, 25]. While the nature of the ligand(s) is not known, the ligation clearly stabilizes Mn(III) against disproportionation to Mn(II) and MnO_2 [25]. Biological ligands such as siderophores can bind Mn(III) , although most siderophores preferentially coordinate Fe(III) , as shown by their overall higher stability constants. However, as described below, some siderophores have a higher affinity for Mn(III) over Fe(III) .

2.1. Desferrioxamine Siderophores

Manganese(III) complexes of the linear and cyclic hydroxamate siderophores desferrioxamine B and E (DFOB and DFOE; Figure 1), respectively, were initially investigated as mimics of superoxide dismutase [26]. A green 1:1 complex of Mn(III)-DFOB (λ_{max} 636 nm, ϵ 128 $\text{M}^{-1}\text{cm}^{-1}$ and 310 nm, ϵ 2050 $\text{M}^{-1}\text{cm}^{-1}$) and Mn(III)-DFOE (λ_{max} 600 nm, ϵ 106 $\text{M}^{-1}\text{cm}^{-1}$ and 325 nm, ϵ 2560 $\text{M}^{-1}\text{cm}^{-1}$) forms quantitatively by air oxidation of Mn(II) in the presence of the siderophores, or by direct reaction of siderophores with Mn(OH)_3 or Mn(OAc)_3 [26]. Equimolar EDTA competes effectively for Mn(III) complexation from Mn(III)-DFOB (i.e., the linear siderophore), but not with Mn(III)-DFOE (i.e., the cyclic siderophore). In the case of Mn(III)-DFOE , a 5-fold excess of EDTA is required to sequester Mn(III) from Mn(III)-DFOE . While these Mn(III) siderophore complexes can catalyze the disproportionation of superoxide, they were less efficient than $\text{Mn}^{2+}_{\text{aq}}$, and thus not efficient SOD mimics [26].

Figure 1: Selected hydroxamic acid siderophores.

The air oxidation of Mn(II) in aqueous solution is pH dependant and facilitated by ligand coordination. Above pH 7, chelated Mn(II) is readily oxidized by dioxygen to Mn(III) [27], and the Mn(III) oxidation state is stabilized by the hard donor oxygen atoms of the hydroxamate ligands [28]. The rate of oxidation of the Mn(II)-DFOB complexes by O₂ is fastest for the fully deprotonated complex, Mn(II)-DFOB⁻, and decreases with increasing state of protonation:

$$k_{\text{Mn(II)-DFOB}^-} = 10.7 \text{ M}^{-1} \text{ s}^{-1}, k_{\text{Mn(II)-HDFOB}^0} = 0.62 \text{ M}^{-1} \text{ s}^{-1}, k_{\text{Mn(II)-H}_2\text{DFOB}^+} = 0.21 \text{ M}^{-1} \text{ s}^{-1} \text{ [27].}$$

The stability constants of the Mn(II) complexes vary with the extent of complex protonation and are all relatively small (i.e., Mn(II)-DFOB⁻, logK = 8.8 ± 0.5; Mn(II)-HDFOB⁰, logK = 7.7 ± 0.4; Mn(II)-H₂DFOB⁺, logK = 5.7 ± 0.4) compared with the Mn(III) complex,

Mn(III)-HDFOB⁺ at $\log K = 29.9 \pm 0.5$ [27]. The stability constant of this Mn(III) complex approaches that of Fe(III) at $\log K = 30.6$ [29]. The Mn(III)-HDFOB⁺ complex is stable from pH 7.1 to 11.3 [27]. Above pH 11.3 Mn(III)-HDFOB⁺ disproportionates, forming a poorly crystalline birnessite precipitate (MnO₂) and Mn(II)-DFOB⁻ [27]. Below pH 7.1 the complex irreversibly decomposes via intermolecular electron transfer, resulting in Mn(II) and oxidized ligand fragments in the 200-400 mass unit range, consistent with hydrolytic cleavage of desferrioxamines, although the specific fragments were not characterized further [27].

Duckworth and Sposito have probed the coordination geometry of Mn(III)-HDFOB⁺ complexes as a function of pH via extended X-ray absorption fine structure (EXAFS) spectroscopy [27, 30-32]. DFOB coordinates to Mn(III) through the fully de-protonated hydroxamate ligands, while the terminal amine remains protonated, giving the complex an overall positive charge [27]. EXAFS fits reveal a Jahn-Teller distorted octahedral geometry wherein the first coordination shell is best split into two distinct environments, one consistent with two elongated axial oxygen atoms and the other with four equatorial oxygen atoms [32].

DFOB also dissolves manganite (γ -MnOOH) through both reductive and non-reductive pathways, depending on pH [31]. Above pH 6.5 the formation of Mn(III)-HDFOB⁺ is observed, while below pH 6.5 aqueous Mn(II) is observed, indicating a reductive pathway, perhaps through oxidation of DFOB by Mn(III), similar to the irreversible decomposition of the Mn(III)-HDFOB⁺ seen below pH 7.1 [27, 31]. Dissolution by DFOB was observed for synthetic and biogenic Mn(IV)-type oxides through a reductive pathway, and hausmannite (Mn₃O₄) through pH-dependent reductive and non-reductive pathways, similar to manganite [31, 33, 34]. Adsorption of Fe(III) from Fe(III)-HDFOB⁺ was also observed in the case of the synthetic and biogenic Mn(IV) type oxides [35]. Because of the ability to bind and dissolve a variety of

minerals, siderophores may play an important role in biogenic cycling of manganese, in addition to iron, and possibly other metals [36-39].

Many other hydroxamate ligands, including other siderophores, such as coprogen, bind to Mn(II) and promote oxidation to Mn(III) [40, 41]. Coprogen (DFC) (Figure 1) is a tris-hydroxamate siderophore that binds Fe(III) and Mn(III) in a hexadentate octahedral coordination similar to DFOB and promotes the oxidation of bound Mn(II) to Mn(III) [40]. Stability constants for Fe(III)-DFC ($\text{Log } \beta_{110} = 30.2$) and Mn(III)-DFC ($\text{Log } \beta_{110} = 28.1$) are both quite high and consistent with the stability constants of other hydroxamic acid siderophores with both Mn(III) and Fe(III) [40, 42].

2.2. Pyoverdinin-type Siderophores

Pyoverdinin-type siderophores were originally isolated from the pseudomonad bacterial family [43]. While pyoverdinin (Figure 2) is a known Fe(III) chelator and is part of a well-studied microbial iron acquisition system, two of its derivatives, pyoverdinin PaA (PVD_{PaA}) and pyoverdinin Gb1 (PVD_{Gb1}) (Figure 2), have higher stability constants for Mn(III) than Fe(III) [28]. The stability constant for $\text{Mn(III)-PVD}_{\text{PaA}}^-$ was determined by spectrophotometric competition between an iron(III)-citrate complex and $\text{Mn(III)-PVD}_{\text{PaA}}^-$ and between $\text{Fe(III)-PVD}_{\text{PaA}}^-$ and a manganese(III)-citrate complex (Equation 2) to approach the equilibrium state from both directions [28] .

The speciation of the ferric and manganic citrate complexes was not described [28], and thus is presented in quotes in Equation 2. The stability constant for $\text{Mn(III)-PVD}_{\text{PaA}}^-$, $\text{log } \beta_{110} = 35.4 \pm 0.2$, is more than five orders of magnitude higher than the ferric complex, $\text{Fe(III)-PVD}_{\text{PaA}}^-$, $\text{log } \beta_{110} = 30.2$.

$\beta_{110} = 30.0$ for [28, 44]. Similarly, the stability constant for Mn(III)-PVD_{Gb1} ($\log \beta_{110} = 35.3$) is higher than Fe(III)-PVD_{Gb1} ($\log \beta_{110} = 31.06$) [28, 45, 46].

The Mn(III)-pyoverdine complexes are consistent with a Jahn-Teller distorted octahedral geometry at Mn(III), as would be expected for a high spin d^4 electronic configuration [28]. The best fit to the EXAFS data for the Mn(III)-pyoverdine complexes is consistent with splitting the first coordination shell into two distinct shells, one with two oxygen atoms in the axial positions and one with four oxygen atoms in the equatorial positions, similar to Mn(III)-desferrioxamine B. The observed preference of these pyoverdines for Mn(III) may be due to conformational changes in the PVD_{Gb1} and PVD_{PaA} backbone which could better accommodate the Jahn-Teller elongation of the axial positions, consistent with high spin d^4 octahedral Mn(III) complexes. Production of PVD_{Gb1} along with other PVD-type siderophores have been linked to regulation of MnO₂ formation and biofilm production [47, 48].

Figure 2: Pyoverdine PaA (top), pyoverdine Gb1 (bottom)

2.3. Rhizoferrin

Rhizoferrin (Figure 3) is a siderophore produced by the fungus *Rhizopus arrhizus* and by the bacterium *Ralstonia picketti* [49, 50]. Although the composition of rhizoferrin from these two sources is the same, the stereochemistry at the chiral quaternary centers is R,R in the case of the fungal *Rhizopus* siderophore and S,S for the *R. picketti* siderophore[50]. Iron(III) coordination occurs through both α -hydroxy carboxylate moieties and pendant carboxylate moieties [49, 50], with a stability constant of $\log \beta_{110} = 25.3$ for Fe(III)-Rhiz³⁻ [51]. Rhizoferrin also coordinates Mn(II) and Mn(III) [28, 51]. The stability constant of Mn(II)-Rhiz⁴⁻, determined by potentiometric titration, is $\log \beta_{110} = 5.95 \pm 0.09$ [28]. The stability constant of Mn(III)-Rhiz³⁻, determined by redox titration and calculated using a modified Nernst equation is $\log \beta_{110} = 29.8 \pm 0.3$ [52], which is substantially higher than the Fe(III) complex. The authors suggest the higher stability constant for Mn(III) may reflect, in part, a stronger interaction of Mn(III) with the hydroxyl on the α -hydroxy carboxylate moieties, as a result of the higher acidity of Mn(III) (pK_a of $\text{Mn}(\text{H}_2\text{O})_6^{3+} \sim 0$) over Fe(III) (pK_a of $\text{Fe}(\text{H}_2\text{O})_6^{3+} \sim 2.7$) [28, 51, 53], although pendant monodentate carboxylate ligation in the axial position (Figure 3) may best accommodate the Jahn-Teller distortion observed by EXAFS of the Mn(III)-Rhiz³⁻ complex [28], in a manner that is not possible with tris bidentate hydroxamate, α -hydroxy carboxylate and catecholate ligands.

Figure 3: Rhizoferrin and Mn(III) complex depicting Jahn-Teller axial elongation

2.4. Protochelin

The tris catechol siderophore protochelin (Figure 6), which is covered below for its role in molybdenum and vanadium acquisition in the nitrogenase-producing bacterium *Azotobacter vinlandii*, also coordinates manganese. The Mn(III)-Proto³⁻ stability constant, $\log \beta_{110}$, is 41.6 which is lower than the Fe(III)-Proto³⁻ stability constant of $\log \beta_{110}$ at 44.6 [54]. While EXAFS data are best fit with four shorter Mn-O equatorial ligands and two longer Mn-O axial ligands, the instability of this complex prompted DFT investigations, which were also consistent with Jahn-Teller distortion at Mn(III). A shift from bidentate catecholate coordination to Fe(III) to salicylate coordination is often observed in catecholamide siderophores, as the pH is lowered [54, 55]. However, the salicylate shift is not observed for Mn(III)-Proto³⁻; instead precipitation is observed below pH 6. Mn(III)-Proto³⁻ reduction to Mn(II) may also occur [54].

Table 1

Selected siderophore/chalkophore stability constants						
Siderophore	Mn(III) $\log\beta_{110}$	Ref.	Fe(III) $\log\beta_{110}$	Ref.	Cu(I) LogK _b	Ref.
DFOB	29.9 ± 0.5	[27]	30.6	[29]		
Coprogen	28.1	[40]	30.2	[42]		
PVD-PaA	35.4 ± 0.2	[28]	30.0	[44]		
PVD-GB-1	35.3	[28]	31.06	[28]		
Rhizoferrin	29.8 ± 0.3	[28]	25.3	[51]		
Protochelin	41.6 ± 0.3	[54]	44.6	[56]		
Mb-Ob3b					20.8	[57]
Mb-Strain M					21.2	[58]
Mb-CSC1					21.1	[58]
Mb-Rosea					20.7	[58]
Mb-SB2					26.9 ± 9.0	[59]

3. Copper Complexation by Chalkophores and Siderophores

Copper plays an important role in many biological systems, primarily in redox active metal centers. Certain bacteria have a high demand for copper, such as the majority of methanotrophs that express the particulate copper methane monooxygenase (pMMO).

Methanotrophs are Gram-negative bacteria that grow on CH₄ oxidation as their sole carbon source. Recently a family of copper-binding chelating agents have been discovered to be produced by methanotrophs. These ligands are usually referred to as methanobactins, but may also be called chalkophores, which derives from their affinity for copper and the Greek word “chalko” for copper.

3.1. Methanobactins

The first methanobactin to be discovered was isolated from the methanotroph *Methylosinus trichosporium* OB3b (Mb-OB3b; Figure 4), which also produces the copper-containing pMMO if sufficient copper is available, or the iron-containing soluble MMO (sMMO) when copper is not available [60]. Methanobactins preferentially bind Cu(I) in a distorted tetrahedral geometry through the nitrogen atoms contained in the two oxazoline rings and two thioamide sulfur atoms [57, 61]. While methanobactin can coordinate Cu(II), it is quickly reduced to Cu(I) through an unknown mechanism [62-66]. The stability constant for Cu(I)-(Mb-OB3b), established by competition with bathocuproine di-sulfonate (BCS) with equilibration established from both directions (i.e., from Cu(I)-Mb + BCS and $[\text{Cu}(\text{BCS})_2]^{3-} + \text{apo-Mb}$) is $\log K = 20.8$ [57]. Uptake of Cu(I)-Mb was demonstrated through isotopic and fluorescent labeling by *M. trichosporium* OB3b, as well as by several other strains of methanotrophs, to occur via an active-transport process [67]. Methanobactins can coordinate a variety of different metal ions, but preferentially bind Cu(I) or Cu(II), and can displace Fe(III) from Mb [68, 69].

Figure 4: Selected methanobactins.

Other methanotrophs (e.g., *Methylococcus capsulatus* Bath and *Methylobacterium album* BG8, *Methylocystis* strain M, *Methylocystis hirsuta* CSC1, *Methylocystis rosea*, *Methylocystis* SB2, etc) produce methanobactins with structures and copper affinities similar to Mb-OB3b (Figure 4; Table 1) [58, 69-71]. The Cu(I) affinity for the *Methylococcus capsulatus* Bath and *Methylobacterium album* BG8 methanobactins were much lower than for Mb-OB3b [69]. Genome mining has recently been employed to search for other methanobactin type natural products in microbes, and has provided evidence that they may be more wide spread than previously thought [72].

3.2. Yersiniabactin

Yersiniabactin (Ybt; Figure 5) is a siderophore produced by pathogenic bacteria, such as *Yersinia pestis* and certain uropathogenic strains of *E. coli* [73-75]. It is now recognized that

both hosts and pathogens vary copper levels at sites of infections [76]. While mammalian hosts often increase Cu levels in response to a bacterial infection, the ability of a pathogen to resist the toxic effects of Cu determines its survival. Uropathogenic strains of *E. coli* secrete Ybt, not only for Fe(III) uptake, but also to chelate Cu(II) as a protective strategy, minimizing the effectiveness of copper as an antimicrobial agent [75-77]. Both Cu(I) and Cu(II) have antibacterial properties, although Cu(I) is considered to be significantly more bactericidal [77].

Figure 5: Yersiniabactin

The Cu(II)-Ybt complex could also provide pathogens protection from the human immune response by mimicking superoxide dismutase activity (SOD) [75, 78]. Having siderophore-based SOD activity may be advantageous over similar enzymatic chemistry because the thiazoline and thiazolidine rings are protease resistant and are therefore only slowly broken down by phagosomes [79].

Coordination of transition metal ions to Ybt is achieved by three nitrogen atoms, one from the thiazolidine ring and two from the thiazoline rings, and three oxygen atoms from a phenolate, a secondary alcohol, and the carboxylate, respectively [80]. While Ybt can bind other metals such as Zn(II) and notably Fe(III) in its role as an iron-transporting siderophore, Cu(II)-Ybt is stable in the presence of Fe(III) and both Fe(III)-Ybt and Cu(II)-Ybt are present in infections at similar concentrations, indicating a possible dual function [77].

4. Molybdenum and Vanadium Complexation by Siderophores

Most nitrogen-fixing bacteria must obtain molybdenum or vanadium, along with iron, to form the active site of nitrogenase [81]. Because of the importance of Mo and V, interest in the mechanism of acquisition of molybdenum and vanadium by *Azotobacter vinelandii* has focused on siderophores, which include aminochelin, azotochelin, protochelin, and azotobactin (Figure 6) [82, 83].

4.1. Protochelin and its Derivatives

Protochelin, azotochelin, and aminochelin (Figure 6) are catechol siderophores that may be regulated by both molybdenum and iron concentrations [82, 84]. Duhme et al showed that when iron and molybdenum are both limited in the growth medium, both aminochelin and azotochelin are produced. As the molybdenum concentration is increased in the growth medium, protochelin is preferentially produced [85, 86]. In a separate study, however, Kraepiel et al have seen that protochelin is produced under most conditions [87, 88]. They also observe reduced production of both protochelin and azotochelin under high molybdenum or vanadium concentrations and uptake of Mo siderophore complexes is shut down under high molybdenum concentrations [87, 88]. While protochelin coordinates to both Fe(III) and MoO_4^{2-} , the molybdenum species appears to be kinetically favored, leading to higher concentration in the supernatant during the lag phase and initial exponential growth phases, while the iron species dominates thereafter [89].

The stability constant for $\text{MoO}_2\text{-Azoto}^{3-}$ ($\text{LogK} = 35$) was determined spectrophotometrically by direct titration of apo-azotochelin with sodium molybdate [85]. Coordination of molybdate (MoO_4^{2-}) by azotochelin occurs through both catechol groups. The octahedral Mo(VI) center is filled out with two oxo ligands [90]. In addition to MoO_4^{2-} ,

protochelin and azotochelin also coordinate vanadate, VO_4^{3-} , and tungstate, WO_4^{2-} . Protochelin coordinates VO_4^{3-} which promotes uptake of vanadium by *A. vinelandii* [87, 88, 91], for presumable incorporation into the alternative nitrogenase. Protochelin also coordinates WO_4^{2-} , and in fact high WO_4^{2-} levels dramatically increases production of protochelin [92] presumably for protective purposes since WO_4^{2-} is toxic to cell growth; minimal uptake of the W(VI)-protochelin complex is observed compared with the Mo(VI)-protochelin complex [92]. Further *A. vinelandii* mutants deficient in catechol siderophore production are much more sensitive to tungstate levels in the environment than wild type *A. vinelandii*.

Figure 6: Catechol siderophores from *A. vinelandii*

4.2. Azotobactin

Azotobactin (Figure 7) is a pyoverdine-type siderophore released in iron limited conditions by *A. vinelandii* [93-95]. In addition to Fe(III), azotobactin binds MoO_4^{2-} and VO_4^{3-} and the resulting complexes are taken up by the bacterium, presumably for incorporation into nitrogenase [96]. Although the binding affinity is much lower for MoO_4^{2-} and $\text{HVO}_4^{2-} / \text{H}_2\text{VO}_4^-$ than Fe(III), the binding of oxoanions may be kinetically favored due to their solubility when

competing for complexation with iron oxide minerals [96], allowing for azotobactin to deliver Mo(VI) and V(V), as well as Fe(III), to the bacterium [96]. Recently it was shown that Mo promotes the expression and buildup of azotobactin during growth of *A. vinelandii* supplemented by 10 μM MoO_4^{2-} [82].

A. vinelandii is an interesting example of a bacterium which has high demands for multiple metal cofactors and utilizes the interplay of different small molecule metal chelators to facilitate uptake of required metal ions. Even in the case of varying metal concentration in the environment, *A. vinelandii* can obtain the iron, molybdenum, and/or vanadium it needs to grow and fix dinitrogen.

Figure 7: Azotobactin δ from *A. vinelandii*

5. Summary

While the vast majority of siderophores are involved in microbial iron acquisition, this review describes siderophores which complex transition metal ions other than iron under physiological conditions. In some cases, the siderophores promote bacterial uptake of these metal ions. Table 1 shows that siderophores with a wide variety of coordinating ligands all have appreciable stability constants for Mn(III), and several siderophores, e.g., PVD_{Gb1} , PVD_{PaA} and rhizoferrin, coordinate Mn(III) with higher affinity than Fe(III). For copper, a new class of microbial ligands

known as methanobactins or chalkophores, have been discovered recently. Genomics predictions suggest chalkophores may be quite common, although to date relatively few have been isolated and structurally characterized. The discovery and characterization of more chalkophores will be quite exciting, including their metal selectivities. Speculation on siderophore-mediated uptake of molybdenum and vanadium has persisted for many decades without conclusive evidence, until recently. Siderophores with only two bidentate ligands, such as the bis-catechol siderophore azotochelin, would seem to be ideal for Mo(VI) and V(V) given the propensity for cis-dioxo or cis oxo-hydroxo coordination in aqueous solution. Molybdate and vanadate uptake assisted by siderophores has now been established, although uncovering the pathways that must govern the competitive uptake of Fe(III) and Mo(V) (or V(V)) will be interesting. Hydroxamate ligands have been used for over a century in colorimetric spot tests to detect Mo(VI) and V(V), thus hydroxamate-siderophore complexation to these oxyanions is a topic of current interest [97, 98]. Putrebactin [99], alcaligin and bisucaberin are all cyclic siderophores with only two hydroxamic ligands, and thus should be good ligands for oxyanions, permitting cis-dioxo or cis-oxo-hydroxo coordination at the metal center. Putrebactin and DFOB bind vanadate and molybdate producing an array of structures varying in nuclearity and state of protonation, depending on the oxoanion concentration and pH [97]. While coordination chemists have been intrigued with coordination of many transition metal ions to siderophores since their initial discovery, future developments to investigate the biological occurrence of ligand-mediated uptake of transition metals other than iron or the expression of ligands to binds metals in virulence strategies will be especially interesting.

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Figure Captions

Figure 1: Selected hydroxamic acid siderophores

Figure 2: Pyoverdine PaA (top), pyoverdine Gb1 (bottom)

Figure 3: Rhizoferrin and Mn(III) complex depicting Jahn-Teller axial elongation

Figure 4: Selected methanobactins.

Figure 5: Yersiniabactin

Figure 6: Catechol siderophores from *A. vinlandii*

Figure 7: Azotobactin δ from *A. vinelandii*