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Biomimetic iron(III) trishydroxamate complexes and triple stranded diferric helices

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Abstract: In order to guarantee sufficient iron(III) uptake, microorganisms produce iron(III) ligands, termed siderophores. They scavenge the scarcely available iron(III) from the environment and deliver it to the cell via a highly specific, active transport process. In this article we describe analogs of hydroxamate siderophores with emphasis on Ferrioxamine derivatives. We present the principles of their design, and synthesis and describe their coordination properties and biological activity. These data enable us to trace the relationship between structural and functional parameters, and apply the knowledge gained for the design of supramolecular arrangements.

INTRODUCTION

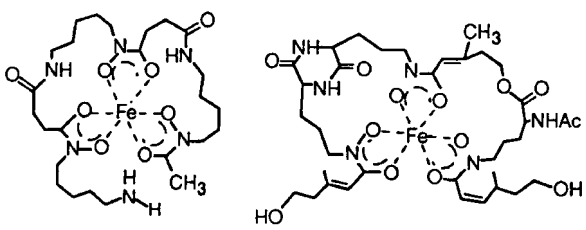
Iron(III) is an essential element for the growth and development of every living cell[1]. Therefore biological systems have developed a variety of powerful tools to guarantee adequate iron(III) uptake. One of these tools are iron(III) ligands or siderophores, that are excreted by microorganisms into the environment where they scavenge the scarcely soluble iron(III)[2-5]. Once loaded with iron(III), the siderophore complexes return to the cells and deliver their cargo to the cytoplasma. A unique feature of this iron(III) delivery process is its high specificity; siderophore-mediated iron(III) uptake is governed by membrane receptors that generally recognize and bind only a single siderophore complex and are capable of chiral discrimination [1,6,7]. Although siderophores may greatly vary in structure, they have a few features in common: (i) the presence of either catechol or hydroxamate groups as iron(III) binding sites, (ii) the capability to generate an octahedral binding cavity for the guest ion and (iii) the capability to generate a molecular envelope that matches the structural requirements of the respective membrane receptors.

Our work in this area is guided by the principles of *biomimetic chemistry*.[8] According to this approach we aim to first identify the essential structural features of the natural siderophores and then reproduce these very features with the simplest possible synthetic molecules. Adopting this approach we prepared bioactive synthetic analogs of the triscatecholate siderophore enterobactin[9] and of the tripodal trishydroxamate siderophore ferrichrome[10,11]. Here we concentrate on synthetic analogs of a third family, the linear trishydroxamate Ferrioxamine[12-14]. We describe these ligands' design and synthesis, examine the ion binding properties and the biological activity and deduce the relationship between structural and functional parameters.

Design and Synthesis

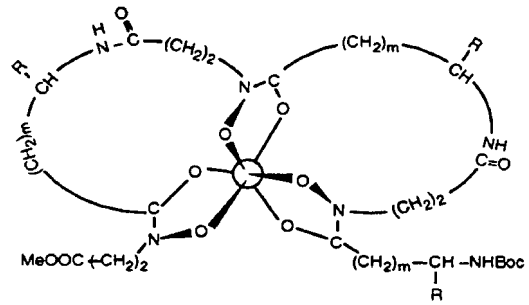
The Ferrioxamines are linear structures[15] that possess three hydroxamate groups which are bridged consecutively by amide- or ester-containing methylene chains. The most studied representative is the linear Ferrioxamine B. Ferrioxamine B can form a total of five conformational isomers when binding trivalent metal ions, each in enantiomeric pairs .[16]

In an attempt to examine the possibility of geometric and chiral discrimination by the Ferrioxamine receptor, we aimed at Ferrioxamine B analogs that would form a smaller number of configurational isomers when binding iron(III). Towards this goal we shortened the bridges between the hydroxamate binding to minimize conformational freedom. In addition, we introduced chiral centers to direct the handedness of the complexes to either the left- or right-handed chiral sense.[12-14]. Adopting these guidelines, we assembled chiral Ferrioxamine analogs from three identical monomers, each composed of a chiral amino acid linked to 3-O-benzyl-hydroxy-amino-propionic acid via a hydroxamate group. The use of amino acids as building blocks enabled systematic modifications and the introduction of asymmetric centers, while greatly facilitating the synthetic procedures. The preparation of the Ferrioxamine analogs was accomplished by coupling the desired amino acid residue with the protected N-hydroxypropionic acid to the monomeric derivative, and subsequent trimerization by the Merrifield method.[12-14].



Ferrioxamine B Coprogen

Figure 1: Trishydroxamate Siderophores: Ferrioxamine B and Coprogen.



Biomimetic Ferrioxamine Analogs

Figure 2: Chemical Structure of Ferrioxamine Analogs; 1, m=0, R=L-Me; 2, m=0, R=L-iBu; 3, m=0, R=L-CH_2CONEt_2; 4, m=2, R=$CONEt_2$

Coordination Properties

The Ferrioxamine analogs were found to bind iron(III) in a 1:1 stoichiometry, and to adopt preferentially Δ-cis configuration when composed of L-amino acids. However, preference for the Δ-configuration was more pronounced in the analogs derived from γ-amino acids than those derived from α-amino acids.[12]

We examined the iron(III) release kinetics of complexes **1-4** with CDTA as sensitive indicators of the complexes' coordination properties[12]. The following mechanism has been proposed for iron release from the four synthetic trishydroxamate complexes and from Ferrioxamine B under our experimental conditions (methanol/water (80:20); T=25.±0.1°C; I=0.1; 5.3≤p[H] ≤6.4) :

$$H^+ + LFc(III) + CDTA \underset{}{\overset{k_1}{\rightleftharpoons}} CDTAFe(III) + L + H^+$$

$$\downarrow k_2 \qquad \text{fast} \downarrow$$

$$LHFe(III)^+ + CDTA \qquad [1]$$

The k_1 values did not show any significant variations in the p[H] range examined. The mono-molecular rate constants k_2 were found to increase linearly with the proton concentrations:

$$k_1 = k_H [H^+] \quad [2]$$

The corresponding rate constants k_1 and k_2 were determined at p[H]=6.3±0.1 and their values are shown in Table 1.

TABLE 1: Kinetic parameters k_1 and k_2; Solvent: methanol/water (80:20); T=25.0 ± 0.1°C; I=0.1; p[H]=6.3±0.1

Complex	$k_1 \pm 2\sigma$ (mol^{-1} Ls^{-1})	$k_2 \pm 2\sigma$ (s^{-1})
1-Fe(III)	± 0.09	$\times 10^{-3}$
2-Fe(III)	± 0.2) $\times 10^{-1}$	$\times 10^{-4}$
3-Fe(III)	± 0.1) $\times 10^{-1}$	$\times 10^{-4}$
4-Fe(III)	± 0.1) $\times 10^{-3}$	
Ferrioxamine B	± 0.6) $\times 10^{-4}$	

The k_H values determined for the ferric complexes **1** and **3** are comparable with the acidic dissociation constants obtained in water for Ferrioxamine B[17,18]($k_H = 3.3 \times 10^2$ mol^{-1} L s^{-1}) or for the tris(acetohydroxamato) iron(III) complex[19] ($k_H = 9.9 \times 10^4$ mol^{-1} L s^{-1}).

1-Fe(III	3-Fe(III)	
k_H (2.0 ± 0.3) $\times 10^3$ mol^{-1} Ls^{-1}	k_H (2.6 ± 0.1) $\times 10^3$ mol^{-1} Ls^{-1}	[3]

These results suggest that the k_2 values determined in this work are related to the acidic dissociation constants observed earlier for the dissociation of the first hydroxamate of Ferrioxamine B[17,18] and other bacterial siderophores.[20] The kinetic measurements revealed that an increase of the length of the spacers between the coordination sites leads to slower ligand exchange rates (about 2 orders of magnitude difference in k_1 between complexes **3** and **4**). Increased bulkiness of the substituents contributes to a significant decrease in the k_1 values, reflecting increased steric hindrance in the ligand exchange mechanism. It is also remarkable to note that the second order rate constant k_1 for the biomimetic ligand **4** is very close to that of the natural Ferrioxamine B (Table 1). The kinetic parameters of the biomimetic ferric complexes thus become comparable with those of the natural Ferrioxamine B as the chain length increases and the strain diminishes.

Biological Activity

All Ferrioxamine analogs possessing a single methylene bridge (m = 0, R = Me, iBu, CH_2CONEt_2), facilitated iron(III) uptake in *Pseudomonas putida*[14,21]. Iron(III) uptake was inhibited by NaN_3, demonstrating its energy dependence. These analogs also competed with both Ferrioxamine B and Coprogen B as iron(III) carriers, indicating their fit to both iron(III) uptake systems. The glutamic acid derivative (m = 2), on the other hand, failed to act as iron(III) carrier, but inhibited Ferrioxamine mediated iron(III) uptake.

Remarkably, the inhibitory effect of the L-glu derivative that forms complexes of Δ-configuration was 2-3 fold smaller than that of the D-glu derivative which forms complexes of Λ-configuration. This demonstrates that the Ferrioxamine receptor exerts chiral discrimination, although the natural Ferrioxamine B is achiral.[21] The activity of the lower homologues (m = 0) as Ferrioxamine and Coprogen analogs (Figure 1), independent on the nature of the amino acid component, is indicative of their fit to both siderophore receptors and transport systems. This may be attributed to the rather small molecular diameters of these iron(III) complexes that can easily adopt themselves to the respective recognition sites. The action of the higher homologues (m = 2) as inhibitors of Ferrioxamine, without affecting Coprogen, suggests higher discrimination by the latter iron(III)uptake system. As for the case of the Ferrichrome receptors, also the Ferrioxamine receptors of different organisms proved to differ in their structural requirements.[21] The Ferrioxamine receptor of *Erwinia herbicola* [13], in contrast to that of *Pseudomonas putida*,[14] proved to be recognized by all synthetic Ferrioxamine analogs, those derived from α–amino acids and those derived from glutamic acid. Species discrimination with synthetic siderophore analogs, either for the purpose of species-specific growth promotion, or for species-specific growth inhibition, thus appears a viable possibility.

Conclusion

Our findings illustrate the role of conformational subtleties in determining *in vivo* activity, indicate the pronounced differences between related siderophore receptors of different organisms, and demonstrate the usefulness of structurally and physiochemically fully characterized synthetic siderophore analogs as probes of microbial iron(III) uptake systems. The lower homologs **1-3** thus fully reproduce the activity of the natural siderophores *in vivo* as growth promoters and iron(III) carriers in two types of microorganisms, *E. herbicola* and *P. putida.*, although they substantially differ from the natural Ferrioxamine B in terms of their coordination properties. The higher homologue **4**, which is very close to Ferrioxamine B in its iron(III) release kinetics, behaves differently in two types of microorganisms, *E. herbicola* and *P. putida.*

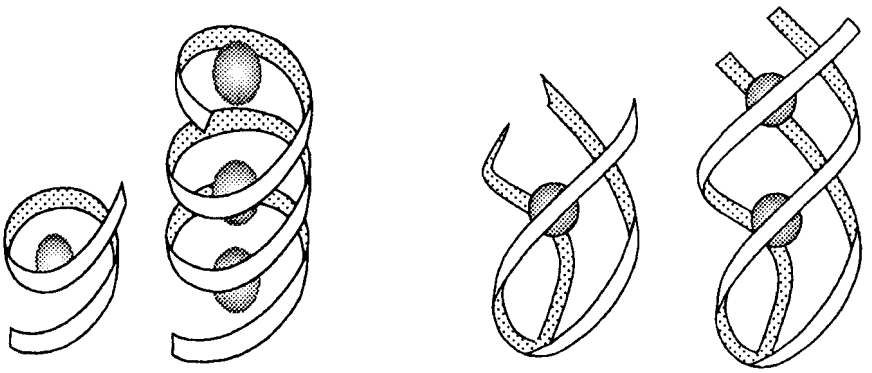


Figure 3: From Siderophores to Supramolecular Structures.
Single stranded helices (left) and triple-stranded helices (right).

Having established the structural parameters that determine iron binding and release properties, the road appears paved towards the generation of supramolecular structures that include multinuclear metal centers. Examples are the generation of helical, triple-stranded, diferric complexes, and of single-stranded di- and tri-ferric complexes (Figure 3). A common theme in these structures is the incorporation of weak-non-covalent interactions, that stabilize these complexes' helical topologies, and the presence of asymmetric carbons that control the helices' chiral sense.

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