Iron(III) Complex of a Trihydroxamate Ligand with an Ala-Ala-(HO)Gly-Ala Sequence as a Model for Ferrioxamines. Formation of Well-Defined Peptide Loop Structure

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A dodecapeptide trihydroxamate ligand forms a well-defined chiral complex with iron(III) in which this small linear peptide assumes a unique loop conformation, uncommon to protein structures. The complex exhibits siderophore activity for a test microorganism.

The design and synthesis of artificially functionalized molecules with peptide frameworks is of interest, since peptides in general provide a diversity of functions when properly formulated.[1] In microbial iron transport systems, siderophores (iron-specific transporting compounds) form stable complexes with iron(III).[2] Desferrioxamines (iron removed ferrioxamines) are a class of naturally abundant trihydroxamate siderophores. Their characteristic structural feature is the presence of a 9-atom spacing between the hydroxamate groups,[3] shown here:

$$-N(OH)CO - (CH_2)_2-CONH-(CH_2)_5 - N(OH)CO-$$

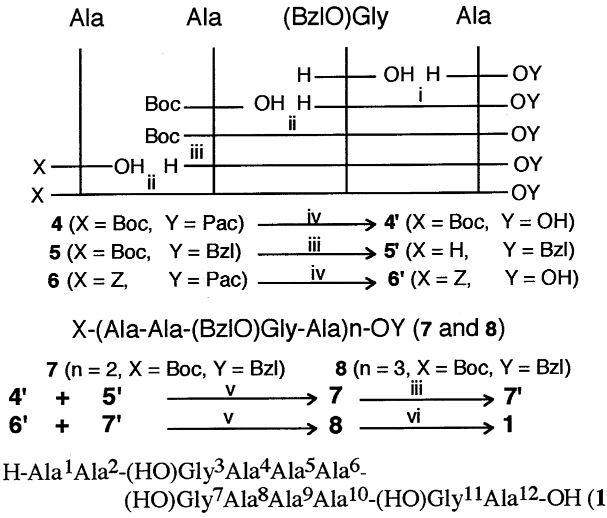
The spacing contains flexible methylene units and allows a ferrioxamine to take several configurations around the metal ion, as evidenced by isomers for Cr(III) desferrioxamine B.[4]

Compared to desferrioxamines, peptides are rigid molecules containing multiple amide groups and possess their own conformational propensity.[1] Therefore, the synthesis of desferrioxamine analogs with peptide frameworks is a challenging subject that requires a basic understanding of the properties of peptides as ligands. Key questions in this respect include: i) what kind of peptide sequences make suitable ligands that form stable complexes, and ii) how do such complexes behave when they are formed?

We report here that a dodecapeptide trihydroxamic acid (**1**) of an Ala-Ala-(HO)Gly-Ala[5] sequence carrying a 10-atom spacing between hydroxamate groups produces a stable iron(III) complex with siderophore activity. The metal complexation forces this small peptide to adopt a unique loop structure[6] not seen among typical peptide conformations.

We previously synthesized a nonapeptide (**2**) with an Ala-(HO)Gly-Ala sequence, which produced an octahedral iron(III) complex (**2**-Fe) only of low stability and weak CD intensity, consisting mainly of the Δ configutration with possible inclusion of the Λ isomer.[7] As related analogs, we have prepared a pentadecapeptide (**3**) of an Ala-Ala-(HO)Gly-Ala-Ala sequence. This trihydroxamic acid produces a 1:2 complex rather than a 1:3 complex of iron(III) to a hydroxamate group. This is ascribed to the conformational constraint of the -$(Ala)_4$- unit in adopting a curved structure. Thus, in the use of L-Ala and (HO)Gly residues, a tetrapeptide sequence like the present Ala-Ala-(HO)Gly-Ala unit seems to be suitable for preparation of peptide trihydroxamate ligands.

The synthesis of the dodecapeptide **1** was carried out by the reactions shown in Scheme 1. Tetrapeptides **4**, **5**, and **6** were joined successively to give octapeptide **7** and dodecapetide **8**.



Scheme 1. *Reagents and Conditions*: i, EDC; ii, Bu^{i}OCOCl and Et_3N in THF; iii, CF_3CO_2H in CH_2Cl_2 and then *N*-methylmorpholine; iv, Zn in AcOH; v,EDC and HOBt in DMF-CH_2Cl_2; vi, H_2 with Pd-C in MeOH.

The final product **1** was purified by gel-chromatography and characterized by HPLC, IR, NMR, and elemental analysis.[8] The presence of the three hydroxamate groups was confirmed by observation of the Ala α-CH protons at δ 4.85 ppm.

A 1:1 molar mixture of iron(III) nitrate and dodecapeptide **1** in water gave a solution of pH 2.1, which contained a 1:2 complex (λ_{max} 460 nm). Neutralization of the solution with alkali produced a 1:3 iron(III) complex (**1**-Fe). Its visible absorption spectrum (not shown) gave a λ_{max} 420 nm with an ε value of 2900, characteristic of a typical 1:3 iron(III) hydroxamate complex.[9] A plot of ε (at 420 nm) vs. pH reveals the plateau region over a pH range of 4.5 to 9.0 (Figure 1), where iron (III) exists exclusively as a 1:3 complex. The ε value is larger and the pH range is wider than those of **2**-Fe (ε =2160 and pH 6.5 - 8.5),[7] reflecting its much increased stability. To obtain the stability constant of **1**-Fe, we determined an equilibrium constant (K_{eq}=

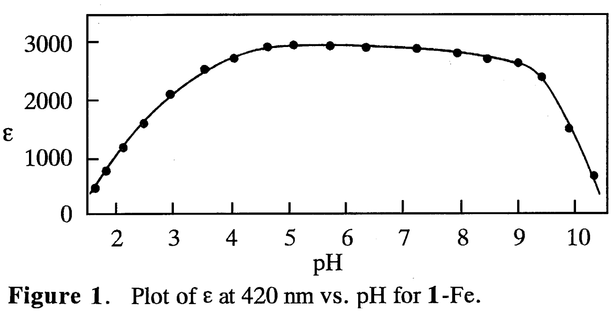


Figure 1. Plot of ε at 420 nm vs. pH for **1**-Fe.

83) for an equimolar ligand-exchange reaction with EDTA (3.2×10^{-4} mol dm^{-3}) at pH 7.4 and the protonation constants (pK_1= 7.48, pK_2= 8.27, pK_3= 8.73) of the three hydroxamate groups, and then we calculated the stability constant to be 6×10^{22}, a small value compared to that of ferrioxamine B (5×10^{30}).[9]

The circular dichroism spectrum of **1**-Fe (Figure 2) shows a positive and a negative band at 365 nm ($\Delta\varepsilon$ = +3.3) and 450 nm ($\Delta\varepsilon$ = -5.4), respectively. These bands are assignable to the Δ configuration around the metal center.[2,10] The $\Delta\varepsilon$ values are much larger than those of **2**-Fe[7] and even larger than those of ferrichrome and coprogen, both well-known natural trihydroxamate iron complexes.[2]

For further examination of the coordination geometry, ^{1}H 2D NMR spectroscopy was investigated, using a separately prepared **1**-Ga(III) complex, a close analog to the iron(III) complex. A combined COSY-NOESY spectrum for amide NH and α–CH protons (δ/ppm) in $(CD_3)_2SO$ at 22 °C gave a single series of connectivity through four disconnected sequences of i) Ala^1-αH(3.93)-Ala^{2}NH(9.07)-αCH(4.82), ii) (HO)Gly$^3\alpha$H(4.48)-Ala^{4}NH(8.26)-αH(4.38)-Ala^{5}NH(8.55)-αH(4.36)-Ala^{6}N(8.60)-αH(4.70), iii) (HO)Gly$^7\alpha$H(4.48)-Ala^{8}NH(8.26)-αH(4.38)-Ala^{9}NH(8.62)-αH(4.45)-Ala^{10}NH(8.60)-αH(5.03), and iv) (HO)Gly$^{11}\alpha$H(5.15)-Ala^{12}NH(8.80)-αH(4.33). Each (HO)Gly residue had its NOE signal only at one of the two α-protons, showing the conformational rigidity of the complex. The observed Ala^4-Ala^5 and Ala^8-Ala^9 NH-NH NOEs indicated the presence of a folded conformation. Notably, this NMR study reveals that two sequences from (HO)Gly^3 to Ala^6 and from (HO)Gly^7 to Ala^{10} have a similar connectivity circuit. This fact further indicates that the two sequences lie in a spatially similar environment, that is, that the three hydroxamato groups orient to the same direction in coordinating to Ga(III).

Examination of CPK molecular models reveals that all-*cis* arrangement of the hydroxamato groups in the Δ configuration produces a molecular structure consistent with the NMR data. However, if a complex takes the Λ configuration, or the other Δ configuration which contains one or more *trans* arrangements, strained molecules of different shapes are produced owing to steric interaction between the (HO)Gly^i CH_2 and the Ala^{i-1} CH_3 groups. The above considerations lead to the conclusion that the Ga(III) complex has the Δ C-*cis, cis* configuration,[2] as depicted for **1**-Fe in Figure 3. The chirality and diminished flexibility compared to desferrioxamines of this peptide ligand permit its complex to take only a limited conformation, resulting in a well-defined loop structure in the **1**-Fe complex.

A growth promotion assay for biological activity was performed by a standard paper disc procedure with water as blank, using *Aerobacterium flavescens* (ATCC 25091), a mutant auxotrophic for hydroxamate siderophores.[11] From the diameter of the hallow of exhibition of growth for **1**-Fe, the activity was found to be nearly half as large as potent ferrioxamine B but larger than previous tripodal hydroxamate complexes.[12] This is in contrast to **2**-Fe which did not show any activity by this test. Thus, it seems that the microbial transport system can tolerate to some extent an iron carrier of a rigid peptide framework. This fact encourages us in our work to synthesize a variety of biomimetic trihydroxamate siderophores that are composed of *N*-hydroxy tetrapeptide units.

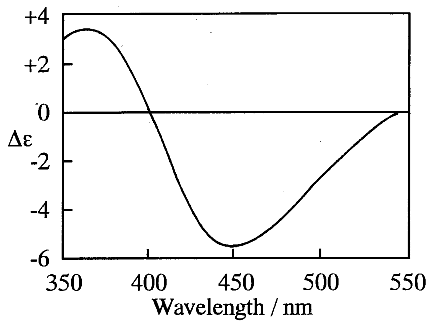


Figure 2. CD spectrum of **1**-Fe in water at pH 7.0.

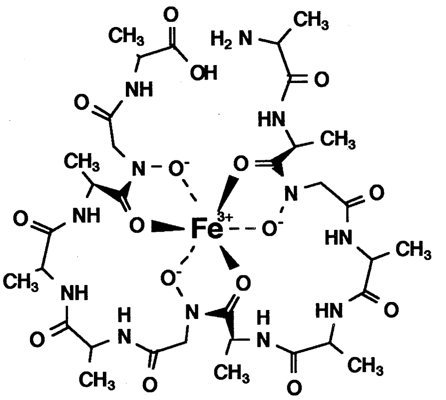


Figure 3. Dodecapeptide iron(III) complex (**1**-Fe) in the Δ C-*cis, cis* configuration.

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References and Notes

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5 Abbreviations: Ala, L-alanine; Boc, *t*-butoxycarbonyl; Bzl, benzyl; EDC, 1-ethyl-3-[(3-dimethylamino)propyl]carbodiimide; EDTA, ethylenediaminetetraacetic acid; HOBt, 1-hydroxybenzotriazole; (HO)Gly or (BnO)Gly, *N*-hydroxy- or *N*-benzyloxyglycine; Pac, phenacyl; Z, benzyloxycarbonyl.

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8 Selected data for **1**, m.p. 204 °C (decomp) (sephadex G-15 with MeOH); $[\alpha]_D$ -128° (H_2O); IR (KBr, ν/cm^{-1}) 1688-1630 (C=O); ^{1}H NMR ($(CD_3)_2SO$ at 40 °C) δ 3.96 (1H, m, α-CH), 4.02 (2H, ABq), 4.14 (4H, ABq), 4.16 (1H, m, α-CH), 4.20-4.35 (4H, m, α-CH), 4.85 (3H, m, α-CH). Found: C, H, N, ± 0.3%. Calcd. for $C_{33}H_{56}N_{12}O_{16}\cdot HCl\cdot 2H_2O$.

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