Notes

Crystal Structure and Conformational Studies of the Ferric Hexadentate *N,N,N*-Tris[2-(3-hydroxy-2-oxo-1,2-dihydropyridin-1-yl)acetamido]ethylamine Complex (FeCP130) as a Specific Iron(III) Chelator

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Introduction

3-Hydroxypyridinones are potential drugs for the treatment of diseases associated with iron-overload.[1] In fact, several bidentate ligands have shown promise in clinical trials.[2] In principle, hexadentate ligands possessing ideal stoeochemistry are stronger iron(III) ligands than their bidentate analogs.[3] This trend has been observed in many siderophores (high-affinity, low-molecular weight iron(III) multidentate ligands).[4,5] Binding experiments, however, have shown that the log K_1 value for iron(III) is 28.8 for FeCP130, which is lower than the log β_3 value for the corresponding bidentate analog (32.3).[1e] This suggests that unfavorable conformational changes are introduced into the hexadentate ligand upon formation of ferric complex FeCP130. For the design of iron(III) chelators, it is essential to explore the molecular conformations for both the ligand and the metal complex since they are responsible for the thermodynamic properties of the chelation process. Herein we present the crystal structure of FeCP130, the first for any hexadentate 3-hydroxypyridinone. The conformational behavior of FeCP130 was investigated both *in vacuo* and in aqueous solvent by molecular mechanics. The results show that the "in" conformation is stable and that the "out" conformation is not. A better understanding of the steric and energetic properties of FeCP130 should provide a basis for the design of superior hexadentate 3-hydroxypyridinones as iron(III) chelators.

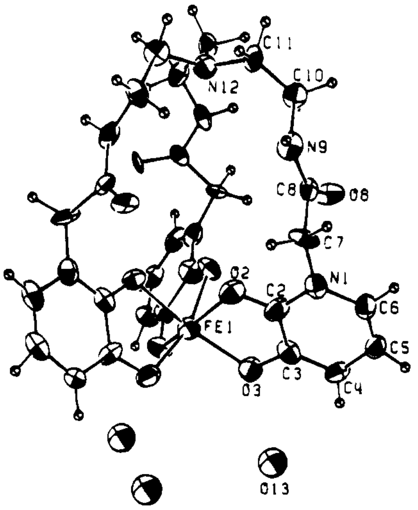

Figure 1. ORTEP plot for the crystal structure of FeCP130.

Table 1. Crystallographic Data for FeCP130

$FeC_{27}H_{30}N_7O_9{\cdot}3H_2O$
fw = 706.47
space group $P4_332$
$a = 19.086(3)$ Å
$V = 6953(2)$ Å^3
$Z = 8$
$\lambda = 0.71073$ Å
$T = 163(2)$ K
$\varrho_{calcd} = 1.36$ g cm^{-3}
$\mu = 51.8$ cm^{-1}
$R = 0.072$
$R_w = 0.077$

Table 2. Selected Bond Distances (Å) and Angles (deg)

	Distances		
Fe–O(2)	2.056(4)	N(1)–C(6)	1.38(2)
Fe–O(3)	1.993(8)	C(2)–C(3)	1.44(2)
C(2)–O(2)	1.26(2)	C(3)–C(4)	1.40(2)
C(3)–O(3)	1.34(1)	C(4)–C(5)	1.41(2)
N(1)–C(2)	1.36(2)	C(5)–C(6)	1.32(2)
	Angles		
Fe–O(2)–C(2)	111.8(8)	O(3)–C(3)–C(2)	114(1)
Fe–O(3)–C(3)	113.8(7)	O(3)–C(3)–C(4)	126(1)
O(2)–Fe–O(3)	80.3(3)	O(8)–C(8)–N(9)	128(1)
O(2)–C(2)–N(1)	122(1)	C(11)–N(12)–C(11′)	114(1)
O(2)–C(2)–C(3)	120(1)		

Experimental Section

Materials and Preparation. The synthesis of ligand CP130 has been described elsewhere.[1e] Complex FeCP130 has been prepared using the method reported by Dionis.[6] The compound was crystallized as dark red blocks from water equilibrated with 1-butanol at 4 °C.

X-ray Crystal Structure Determination. The data crystal of 0.15 × 0.15 × 0.20 mm was mounted on an Enraf-Nonius CAD-4 diffractometer. Information pertinent to data collection and refinement are listed in Table 1. Systematically absent reflections ($h00$, $h \neq 4n$) were consistent with space groups $P4_132$ and $P4_332$. Since the ligand is achiral, one can expect the chelate to be a mixture of equal amounts of the Λ and Δ configurations for the metal environment. The data crystal was proved by the R-factor ratio test ($R = wR_+/wR_-$) to have the Λ configuration, space group $P4_332$. Absorption corrections were made.[7] Only 40% of the unique data were observed although the data crystal was not particularly small. This was attributed to the disorder of water molecules in the structure. The structure was solved by Patterson methods using SHELXS86[8] and refined by full-matrix least-squares minimization of $\sum w(|F_o| - |kF_c|)^2$, where $w = 1/[\sigma^2(F) + 0.0005F_o^2]$, using SHELX76.[9] All shift/esd ratios were less than 0.02,

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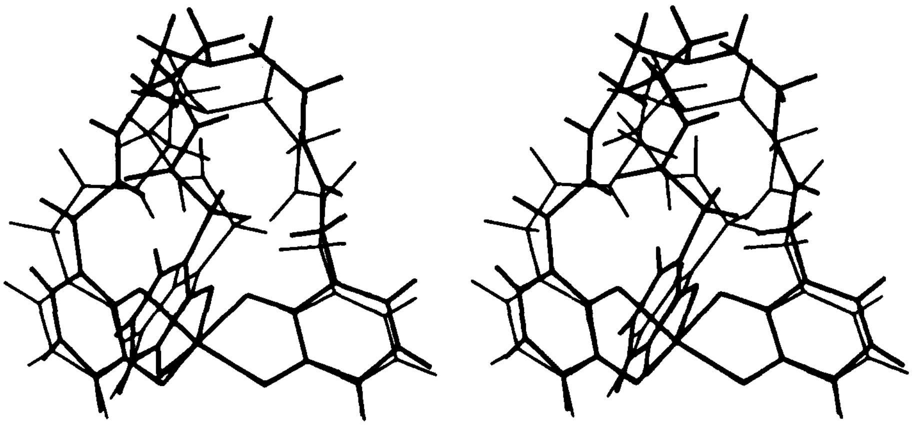

Figure 2. Stereoview of the energy minimization structure (thin line) and the crystal structure (thick line) for FeCP130.

and the final difference density map showed a maximum peak of about 0.62 e/Å^3 near the Fe atom.

Computational Procedures. Molecular mechanics (MM) calculations were performed with the program AMBER4.[10] An ionic model has been employed in modeling the iron(III) coordination. The parameters for intramolecular and nonbonded intermolecular interactions have been taken from the AMBER4 database, except those for iron(III), since they are unavailable. The atomic charge for iron(III) was taken from that of aluminum(III) and this has been fully discussed elsewhere.[11] The well depth of iron(III) Lennard-Jones potential was taken from the universal force field (UFF)[12] while the van der Waals radius was adjusted to reproduce the Fe–O bond distances as observed by X-ray diffraction. The crystal structure serves as the starting geometry. The atomic charges were calculated by the MNDO method with ESP fitting, using the program MOPAC5.[13] They were multiplied by 1.42, which scales MNDO/ESP charges to the *ab initio* (6-31G*) values.[14] For water the TIP3P charges were used.[15] The 1,4 electrostatic and van der Waals interactions have been scaled by a factor of 0.5 (p 81, ref 10). The cutoff for nonbonded interactions was set at 15 Å. To avoid excessive distortion of the iron coordination geometry, positional restraints were imposed with respect to the coordination geometry as observed in the crystal structure. A value of 100 kcal/mol was used for the harmonic restraint force constants. Energy minimization was first carried out for FeCP130 in the gas phase without boundary conditions. The molecule was subsequently placed in a box of water molecules to allow for intermolecular hydrogen bonding. This box was surrounded by 26 boxes of aqueous solvent. Interactions beyond 10 Å from any atom were ignored. An energy minimization was assumed to have converged if the norm of the energy gradient was less than 0.0001 kcal/mol Å.

Results and Discussion

In the crystal structure (Figure 1), the Fe and tertiary amine N(12) atoms occupy positions on the 3-fold axis. As a result, the asymmetric unit contains one-third of the molecule. The molecule adopts the "in" conformation induced by the tripodal tertiary amine N(12). The amide group forms an intermolecular H-bond with the carbonyl O(8) from a neighboring molecule (2.85(2)Å) while the deprotonated hydroxyl O(3) forms an H-bond with water (2.96(2) Å). The water sites were partially occupied with occupancy factor of 0.4. Selected geometric parameters are listed in Table 2. They compare well with those for ferric tris(1-butyl-3-hydroxy-2(1*H*)-pyridinone),[1d] a bidentate analog, despite the large esd's in FeCP130. The determined bond lengths along the chains in FeCP130 deviate from their corresponding standard values.[16] These deviations were attributed to the poor quality of the crystal.

In the energy-minimized structure, the iron(III) coordination geometry observed in the crystal structure was reproduced quite well (Figure 2) and the "in" conformation was preserved. There is, however, a drastic conformational change for the three amide groups, forming intra- rather than intermolecular H-bonds with the three chelating carbonyl O(2) atoms, resulting in seven-membered rings. In the energy-minimized structure for the most stable siderophore iron(III) complex, ferric enterobactin, the amide groups form H-bonds with the chelating hydroxyl oxygens, giving six-membered rings.[17]

The crystal structure and energy minimization both demonstrate the existence of the "in" conformation. Either the "out" conformation is less stable than the "in" conformation or there is a large energy barrier separating these two conformations. The "out" conformation was constructed by flipping the tripodal amine N(12) atom out and subsequently subjecting the resulting structure to energy minimization. The result of this calculation was that the structure was driven back to the identical "in" conformation obtained previously. However, positional restraints on the tripodal N(12) and its three connecting C(11) atoms preserved the "out" conformation after energy minimization. The main conformational differences between the energy-minimized "in" and "out" structures (Figure 3) occur between the amide N(9) and the tripodal N(12) while the intramolecular H-bonding pattern remains unchanged.

The total energy of the system was calculated to be higher for the "out" conformation than for the "in" conformation by 5.2 kcal/mol. The contribution of the electrostatic interactions contributes very large negative values in both conformations, but the difference of this term is small (1.3 kcal/mol). The strain energy, however, is significantly lower in the "in" conformation than in the "out" conformation, the difference being 5.8 kcal/mol. This molecular strain derives from an unfavorable conformational change increasing bond angles N(9)–C(10)–C(11), C(10)–C(11)–N(12), and C(11)–N(12)–C(11)′ in the "out" conformation by an average of 4° from the ideal geometry. This is not the case in the results for the "in" conformation.

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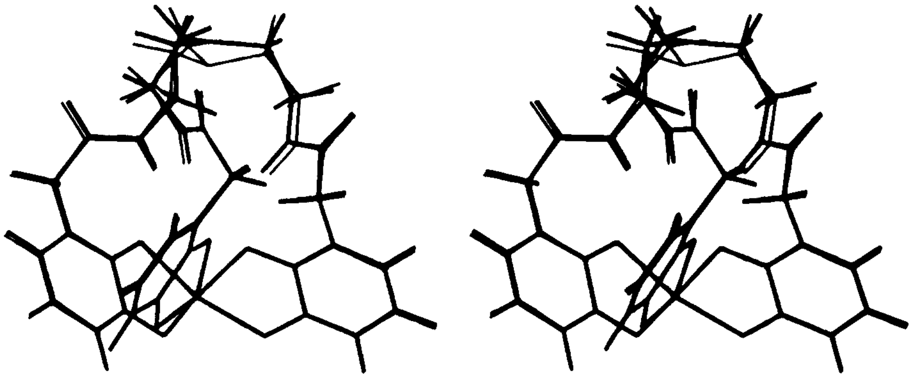

Figure 3. Stereoview of the energy-minimized "in" (thin line) and "out" (thick line) conformations for FeCP130.

Also, the "out" conformation shows 50% more van der Waals contacts and this may be correlated with the observed increases in bond angles. These arguments may well explain the fact that most tripodal metal complexes observed in their crystal structures adopt the "in" conformation.[18]

The "in" conformation *in vacuo* served as a starting geometry for energy minimization in aqueous solvent. Important features of the molecule, such as the intramolecular H-bonding pattern and the position of the tripodal N(12), are preserved in the aqueous solution. In the solid state structure, the amide groups in FeCP130 are the only donors available for the carbonyl O(8) to form H-bonds whereas, in solution, water molecules can serve as either H-bond donor or H-bond acceptor. This allows FeCP130 to preserve its relatively strong intramolecular H-bonds in aqueous solvent while enabling the carbonyl O(8) to form H-bonds with water molecules (2.83 Å). Consequently, molecule FeCP 130 has used its full potential to form H-bonds and hence is stablized in aqueous solvent. In the aqueous modeling, the "out" conformation is unstable. FeCP130 is, therefore, predicted to adopt similar conformations both *in vacuo* and in aqueous solvent; this conformation is however different from the one observed in the solid state.

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Supplementary Material Available: Tables listing data collection parameters, atomic coordinates, bond lengths and angles, anisotropic thermal parameters, H atom coordinates, and torsional angles (6 pages). Ordering information is given on any current masthead page. Observed and calculated structure factors are available directly from the corresponding author.

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