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A structural model of the ferrichrome type siderophore: chiral preference induced by intramolecular hydrogen bonding networks in ferric trihydroxamate†

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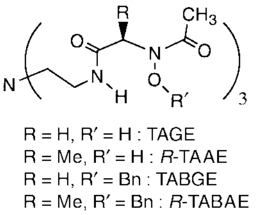
Tris{2-[(*N*-acetyl-*N*-hydroxy)-D-alanylamino]ethyl}amine (*R*-TAAE) has been synthesized as a chiral trihydroxamate artificial siderophore with hydrogen bonding networks, whose crystal structure of the iron(III) complex revealed Λ configuration induced by interstrand hydrogen bonding networks and steric repulsion by optically active amino acid residues.

Siderophores are low molecular weight iron(III)-sequestering agents produced by bacteria and fungi that form very stable iron(III) complexes.[1,2] A large number of siderophores form chiral complexes, because the ferric siderophore complexes are absorbed by microorganisms through specific receptors that recognize the absolute configuration of complexes on the cell membrane.[1,2] The absolute configurations of iron(III)–siderophore complexes have been determined *via* X-ray crystallography and circular dichroism (CD). For example, ferrichrome, a trishydroxamate siderophore, and enterobactin, a triscatecholate iron carrier, form complexes with a left-handed (Λ) and right-handed (Δ) helicity, respectively.[3–5] The chirality of siderophore–iron complexes differs, although they are induced by the same optically active L-amino acid. In order to examine the factors responsible for determining the chirality of siderophores, many analogues have been synthesized. Shanzer and coworkers have reported that intramolecular hydrogen bonds in chiral siderophore analogues are important for the chiral preference and stability of complexes.[6] However, crystal structures of chiral siderophore analogue complexes with such hydrogen bonds have not been reported yet. Recently, we reported a crystal structure of a racemic mixture of iron(III)–tris{2-[(*N*-acetyl-*N*-hydroxy)glycylamino]ethyl}-amine (TAGE, Scheme 1) and observed a triple-stranded helix with both *intra- and interstrand* hydrogen bonding networks.[7] Such a triple helix structure is expected to induce a pronounced difference between diastereomers by introduction of a sterically hindered chiral group. Next, tris{2-[(*N*-acetyl-*N*-hydroxy)-D-alanylamino]ethyl}amine (*R*-TAAE, Scheme 1) was synthesized as a chiral trihydroxamate artificial siderophore, where chiral centers are introduced in the TAGE ligand by replacing the Gly residues with D-Ala residues. Here, on the basis of the crystal structure and CD data of the iron(III) complex with *R*-TAAE, we describe the influence of the intramolecular hydrogen bonding networks on the chiral preference of the iron(III) complex of artificial siderophores.



Scheme 1 TREN based trihydroxamate ligands with hydrogen bonding networks.

The ligand *R*-TAAE was synthesized according to a modification of a previously published method.[8] The iron(III) complex of *R*-TAAE (**1**) was prepared by reaction of Fe(acetylacetonato)$_3$ with *R*-TAAE in a biphasic ethyl acetate–water solution.[9] The crude complex was dissolved in an ethyl acetate solution containing a small amount of methanol, and then after slowly concentrating the solution for a few weeks, a deep-red colored single crystal of **1** suitable for X-ray diffraction analysis was obtained.‡

Initially the space group of the single crystal of **1** could not be distinguished from either $P4_12_12$ or $P4_32_12$ due to the enantiomorphous relationship between the two space groups. From the configuration of the ligand (*R*), the anomalous dispersion effect with Cu Kα radiation, and the difference in R_w values for Λ(*R*,*R*,*R*) and Δ (*S*,*S*,*S*) forms, 3.2 and 8.5, respectively, the space group and the absolute configuration of **1** were eventually determined to be $P4_12_12$ and Λ(*R*,*R*,*R*), respectively. The absolute configuration of **1** is identical to that of ferrichrome, although the chirality of the ligand differs from ferrichrome with Λ(*S*,*S*,*S*). As shown in Fig. 1, the iron ion has a distorted octahedral geometry with three hydroxamates and the overall structure is twisted with a pseudo-3-fold axis.

The average bond lengths between the iron(III) atom and the coordinating *N*-hydroxy O(N) and carbonyl O(C) atoms are Fe–O(N) = 1.970(7) and Fe–O(C) = 2.035(8) Å. The average

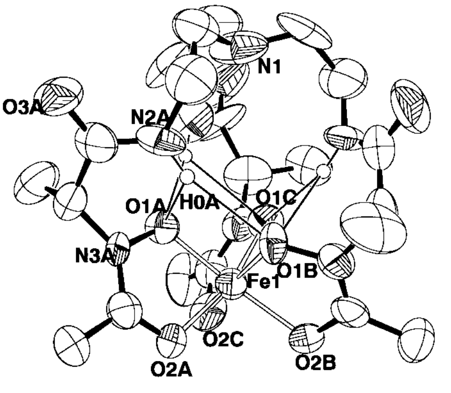


Fig. 1 Crystal structure of **1** showing the atom numbering schemes. Selected bond lengths (Å) and angles (°): Fe–O(1A) = 1.973(7), Fe–O(1B) = 1.964(7), Fe–O(1C) = 1.973(8), Fe–O(2A) = 2.029(8), Fe–O(2B) = 2.038(8), Fe–O(2C) = 2.037(8), O(1A)–Fe–O(2A) = 78.8(3), O(1B)–Fe–O(2B) = 78.8(3), O(1C)–Fe–O(2C) = 79.0(3). Intra- and interstrand hydrogen bond distances (Å): N(2A)···O(1A) = 2.78(1), N(2B)···O(1B) = 2.82(1), N(2C)···O(1C) = 2.92(1), N(2A)···O(1B) = 3.25(1), N(2B)···O(1C) = 3.17(1), N(2C)···O(1A) = 3.12(1).

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† Electronic supplementary information (ESI) available: UV-vis and CD spectra of **1** and details of the determination of the absolute configuration and the space group of **1** are deposited as supporting information. See http://www.rsc.org/suppdata/cc/b1/b100306m/

O(N)–Fe–O(C) bond angle is 78.9(3)°. These values are quite comparable to those for natural trihydroxamate siderophores[2,3] and Fe^{III}TAGE.[7] The twist angle[10] determined for **1** is 44.0°. This is slightly larger than those of natural siderophores (ferrichrome = 42.5, ferricrocin = 40.4°)[2d] and is comparable to the calculated value (45.7°). As the difference between the observed and calculated twist angles is caused by deviation from the idealized C_3 symmetry,[2d] these findings reflect the difference in the symmetries of ligands. The average distances between amide nitrogen and coordinating *N*-hydroxy oxygens are 2.84 and 3.18 Å between intrastrands and interstrands, respectively. These values are slightly shorter than those observed for Fe^{III}TAGE (2.88 and 3.20 Å),[7] indicating that the intramolecular hydrogen bonding networks are enhanced by the introduction of optically active D-Ala to the ligand TAGE. The formation of such a strong intramolecular hydrogen bond is also supported by the ^1H NMR data of the benzyl-protected precursor of *R*-TAAE (*R*-TABAE, Scheme 1). The NMR data revealed a pronounced anisotropy in the benzyl protons $ArCH_2$ and tris(2-aminoethyl)amine (TREN) backbone protons NCH_2CH_2NH in $CDCl_3$ but not in DMSO-d_6. This anisotropy is evidently due to the restriction of rotation induced by intramolecular hydrogen bonding networks.[6b] On the other hand, such anisotropy was not observed for TABGE (a similar precursor of TAGE, Scheme 1) in both $CDCl_3$ and DMSO-d_6.

In aqueous solution at pH 7.4, the UV-vis spectrum of **1** showed a characteristic absorption band at 420 nm corresponding to the tris(hydroxamato)iron(III) complex. This indicates that the structure of **1** determined by X-ray crystallography is maintained in an aqueous solution. The CD spectrum of **1** exhibited positive and negative Cotton effects at 444 ($\Delta\varepsilon$ = +1.0) and 358 nm ($\Delta\varepsilon = -0.8$). These values are qualitatively similar to those determined from the solution and crystalline CD spectra of ferrichrome,[3] indicating that, like ferrichrome, the absolute configuration of **1** is Λ in both the solution and solid states.

Consequently, the observed chiral preference of **1**, crystalline and in solution, is reasonably explained as a result of the orientation of the triple helix induced by intramolecular hydrogen bonding networks becoming tightly fixed by steric repulsion between optically active amino acid residues and terminal methyl groups. This result strongly supports the proposal by Shanzer *et al.*[6] In addition, it is revealed that intramolecular hydrogen bonding networks in the benzyl-protected precursor of *R*-TAAE and its iron(III) complex are easily formed due to the existence of an asymmetric site within the hydrogen bonded six-membered ring. These observations suggest that both intramolecular hydrogen bonding networks and optically active amino acid residues operate in concert, which play an important role for the chiral preference of artificial siderophore complex **1**. The bioavailability of **1** was also investigated using *Microbacterium flavescens* which has ferrichrome receptors but lacks the ability to produce siderophores. Interestingly, complex **1** promoted the growth of this mutant. Therefore, the Fe^{III}*R*-TAAE complex, which has structural features that are similar to those of ferrichrome, might be a good functional model compound for the ferrichrome-type siderophore. Detailed investigations are now under way.

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Notes and references

‡ *Crystal data*: $C_{21}H_{36}N_7O_9Fe$, M = 586.40, tetragonal, a = 17.237(3), c = 19.432(2) Å, U = 5733(1) Å^3, T = 296 K, space group $P4_12_12$ (no. 92), Z = 8, D_c = 1.349 g cm^{-3}, μ(Cu-Kα) = 46.94 cm^{-1}, F(000) = 2472.0, R = 0.053, R_W = 0.032. A total of 2519 unique reflections (R_{int} = 0.102) were collected on a Rigaku AFC5R diffractometer with graphite monochromated Cu-Kα radiation and a rotating anode generator, of which 1585 reflections with $I > 3\sigma(I_o)$ were used in the structure analysis and refinement using the *teXsan* program system. Absorption correction was applied. The absolute configuration and the space group of the complex were determined by comparison of observed and calculated Bijvoet pair differences, whose details are deposited as supporting information. CCDC 158411. See http://www.rsc.org/suppdata/cc/b1/b100306m/ for crystallographic files in. cif format.

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10 The twist angle is defined by the O(N)–Fe–O(C) angle of a single hydroxamate ligand projected onto the plane perpendicular to the idealized 3-fold axis.