Crystal Structure and Solution Behavior of the Iron(III) Complex of the Artificial Trihydroxamate Siderophore with a Tris(3-aminopropyl)amine Backbone

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Tris[{3-(*N*-acetyl-*N*-hydroxy)-glycylamino}propyl]amine (TAGP) forms a 1:1 tris(hydroxamato)iron(III) complex even at a low pH(≈2), which promotes the growth of the siderophore-auxotrophic mutant *Microbacterium flavescens*.

Introduction

Microorganisms produce low molecular weight compounds called siderophores for an uptake of iron. A large number of siderophores have hydroxamates or catecholates as the iron binding site that exhibit very high affinities for iron(III), and thus form very stable iron(III) complexes.[1,2] The high stability of Fe^{III}-siderophore complexes is attributed to not only such chelating effects but also to hydrogen bonding, van der Waals interactions and the predisposition of the ligands.[3,4] Thus, the ligand backbones are quite important for the stabilization of iron(III) complexes. Many artificial tripodal siderophores have been synthesized as ferrichrome and enterobactin analogues; the synthetic siderophores possess various tripodal backbones such as follows tris(2-aminoethyl)amine (TREN),[5] triaminomethylbenzene,[6] 1,5,9-triazacyclododecane,[7] nitrilotriacetic acid,[8] and 1,1,1-tris[(2-carboxyethoxy)methyl]propane.[5] Among such tripodal backbones, TREN has been used most frequently. Recently, we have reported the synthesis of tris{2-[(*N*-acetyl-*N*-hydroxy)glycylamino]ethyl}amine (TAGE, Figure 1) as an artificial siderophore with a TREN anchor; its iron(III) complex showing an extremely high stability ($\log\beta = 28.7$).[5c] From the crystal structure of the iron(III) complex,[5c] this high stability was found to be due to the intramolecular hydrogen-bonding networks between amide hydrogens and coordinating *N*-hydroxy oxygens in the intra- and interstrands. However, tripodal anchors such as TREN have also been reported to form less-stable iron(III) complexes than the natural siderophore-iron(III) complexes, presumably due to the small size of the anchor for iron(III) chelation.[9]

Tris(3-aminopropyl)amine (TRPN), whose alkyl chains are one methylene group longer than TREN, has not been used much despite the similarities in its structure with TREN. We therefore synthesized tris{[3-(*N*-acetyl-*N*-hydroxy)glycylamino]propyl}amine (TAGP, Figure 1) as a trihydroxamate artificial siderophore with a TRPN anchor in order to examine the effect of the size. Here, we will describe the crystal structure and solution behavior of its iron(III) complex.

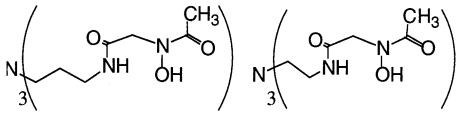

Figure 1. Ligands used in this study

Results and Discussion

As shown in Figure 2, the iron(III)-TAGP complex (**1**) was isolated as a hydrochloride salt with the tertiary amine nitrogen protonated. The quaternary amine is in the "out"

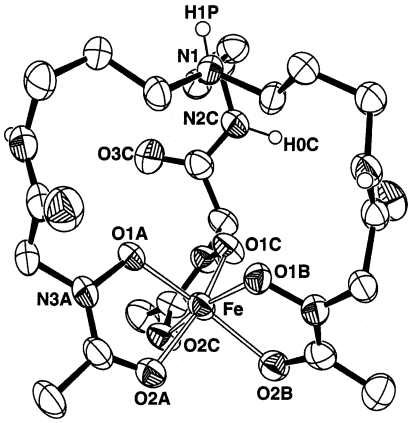

Figure 2. Crystal structure of **1**, showing the atom numbering schemes; selected bond lengths (Å) and angles (deg): Fe−O(1A) 1.971(4), Fe−O(1B) 1.992(3), Fe−O(1C) 1.967(3), Fe−O(2A) 2.036(3), Fe−O(2B) 2.081(3), Fe−O(2C) 2.041(4); O(1A)−Fe−O(2A) 79.3(1), O(1B)−Fe−O(2B) 77.6(1), O(1C)−Fe−O(2C) 78.6(1); all hydrogen atoms except for the amide and TRPN amine hydrogens and the counter anion have been omitted for clarity

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conformation, which is in contrast to many iron(III) complexes with synthetic siderophores that are in the "in" conformation.[5c−5e] The iron(III) ion has a distorted octahedral geometry with three coordinated hydroxamates, and the overall structure is twisted with a pseudo-threefold axis. This crystal structure is very similar to the calculated lowest energy conformation of the iron(III) complex of the trishydroxamate with a triscarboxylate anchor that has previously been reported by Shanzer et al.[3b]

The average bond lengths between the iron(III) atom and the coordinating *N*-hydroxyl O(1) and carbonyl O(2) atoms are 1.977(3) and 2.053(3) Å, respectively, and the average O(1)−Fe−O(2) bond angle is 78.4(1)°. The average Fe−O(2) bond length is 0.01−0.02 Å longer than those of natural and artificial trihydroxamate siderophores (2.022−2.041 Å);[2][5c] this is due to the longer Fe−O(2B) bond (0.04−0.05 Å longer than normal). The rest of the Fe−O bonds are very similar to those for other iron(III)-siderophore complexes.[2][5c] The twist angle[10] determined for **1** is 43.9°, which is comparable to the calculated value (45.7°). Considering the definition of the twist angle, these findings suggest that the configuration around the metal ion is nearly the idealized C_3 symmetry.

The distance between the iron(III) ion and the quaternary amine nitrogen in **1** is 5.205 Å, which is only slightly longer than that in Fe^{III}TAGE ($Fe\cdots N_{amine}$ = 5.139 Å). In the catechol macrobicyclic ligand-metal complexes Na_3[Fe(BCT)] and Na_3[Fe(BCTPT)],[11a] the apical N−N distance between the two TRPN caps {Na_3[Fe(BCTPT)]: 10.70 Å} is longer than that in TREN {Na_3[Fe(BCT)]: 9.76 Å}.[11b] Thus, the short distance between the iron(III) ion and the amine nitrogen in Fe^{III}TAGP may be attributed to the electrostatic interaction between the protonated amines and the hydroxamate anions. The steric restriction of the TRPN anchor may be also reduced by adopting a configuration such that the iron(III) ion is wrapped into the TAGP ligand.

The TAGP amine proton forms a hydrogen bond with a chloride ion on the pseudo-threefold axis [N(1)···Cl(1) = 3.081(4) Å]. In the crystal, the amide groups form intermolecular hydrogen bonds between the nearest neighbor molecules [N(2B)···O(1B) = 3.070(5), N(2B)···O(2A)= 3.099(5) and N(2C)···O(3B) = 3.055(6) Å] and between the complex and the water molecules [N(2A)···O(1 W) = 2.838(8) and N(2C)···O(2 W) = 3.134(7) Å]. The intramolecular hydrogen bond found in Fe^{III}TAGE,[5c] however, was not observed.

The UV/Vis spectra of iron(III)-TAGP complex measured in various pH conditions showed a characteristic absorption band corresponding to an LMCT at 425 nm, which did not show a large spectral change over a wide pH range of 2−8 (Figure 3), indicating that TAGP forms a stable tris(hydroxamato)iron(III) complex even under strongly acidic conditions. However, the iron(III)-TAGE complex exhibited the same characteristic absorption band at only pH 4−8 in aqueous solution. The difference in the formation behaviors of the iron(III) complexes with TAGP and TAGE under acidic conditions is probably due to the different conformations of the tertiary amine, "in" and "out". That is, as the tertiary amine in Fe^{III}-TAGE complex takes the "in" conformation and is protected from the outer sphere by intramolecular hydrogen bonding networks,[5c] the tertiary amine is deprotonated, which probably contributes to the formation of the stable tris(hydroxamato)-type complex. In contrast, as the tertiary amine in the Fe^{III}-TAGP complex takes the "out" conformation, the protonated tertiary amine does not affect the formation of the complex. Hence, the stable tris(hydroxamato)-type Fe^{III}-TAGP complex is formed even under acidic conditions.

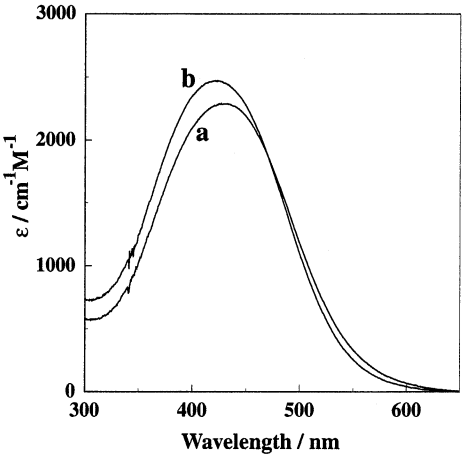

Figure 3. UV/Vis spectra of iron(III)-TAGP complexes (0.1 mM) in an aqueous solution (I = 0.1 M $NaClO_4$): (a) pH 2.0, (b) pH 7.4

The pK_a of the tertiary amine in TAGP is 7.61,[12] which is larger than that of TAGE (pK_a = 5.46), suggesting that the Fe^{III}-TAGP complex is protonated even in a neutral aqueous solution. Comparing the formation constants of the complexes, the protonated Fe^{III}-TAGP complex (log β_{111} = 30.6[12]) is more stable than the Fe^{III}-TAGE complex (log β_{110} = 28.7),[5c] and is similar to the natural siderophore ferrioxamine B (log β_{111} = 30.6).[13] These results indicate that the TRPN anchor brings about a larger stabilization of the complex than TREN, which is one of the important factors of the siderophore function. In contrast, the ferrichrome analogue with a C-terminated triscarboxylate anchor, Et-C($CH_2OCH_2CONHCH_2CONOHCH_3$)$_3$, which was previously reported by Dayan et al., forms polymeric and polynuclear complexes rather than a 1:1 Fe^{III}-ligand complex; this was explained in terms of the absence of stabilization factor such as intramolecular noncovalent interactions.[3a] In spite of the high structural similarity between our ligands and theirs, the complexation behaviors are very different. The substantial difference between the ligands is that the terminal atom of the tripodal ligand is either an amine nitrogen or an alkyl carbon. Thus, the high stability of the iron(III)-TAGP complex identified in the wide pH range of 2−8 is achieved by the electrostatic interaction between the positively charged TRPN anchor and the negatively charged aminohydoxyl oxygens.

The bioavailability of **1** was also investigated using *Microbacterium flavescens* (ATCC No. 25091), which cannot synthesize siderophores by itself. Although the growth of the strain was not observed in the siderophore-free ATCC No. 424 broth,[14] it was seen in the medium containing TAGP. This result indicates that TAGP has a siderophore function transporting an iron ion from the medium into the cell. A similar compound, TAGE, also promoted growth of the same strain under the same conditions; the difference between the TREN and TRPN anchors was not observed. However, the above-mentioned ferrichrome analogues with a C-terminated triscarboxylate anchor did not promote the growth of the siderophore-auxotrophic mutants of *Pseudomonas putida*,[3a,3c] although the strains examined are different from those which were used in this study. The microbial inactivity of these complexes is either because of the formation of polymeric and polynuclear complexes, or because the 1:1 Fe^{III}-ligand complexes, if they are formed, take a conformation that inhibits the receptor binding.[3a] As mentioned above, the substantial difference between **1** and these complexes is that the complex either has a positive charge or it does not; the former has a positive charge but the latter are neutral. Therefore, a positive charge on a complex may contribute to the promotion of biological activity.

The above findings suggest not only the availability of TRPN as an anchor in the siderophore function but also the biological implication of electrostatic interactions in the positively charged natural siderophores such as desferrioxamine B. Detailed investigations are in progress.

Experimental Section

Fe^{III}TAGP·HCl (1): The ligand TAGP was synthesized according to a modification of a previously published method.[15] The iron(III) complex of TAGP was prepared by the following method. A few drops of pyridine were added to a methanol solution containing $FeCl_3$ and TAGP in a 1:1 molar ratio.[16] Slow evaporation of the solution for a few weeks at room temperature gave a deep-red colored single crystal of **1** suitable for X-ray diffraction analysis.[17]

X-ray Crystallographic Study: Crystal data for $C_{22}H_{45}ClFeN_7O_{12}$ (molecular weight = 690.04): triclinic system with cell dimensions at 288 K of a = 11.711(7), b = 13.285(4) c = 10.974(6) Å, α = 93.04(5), β = 102.56(3), γ = 101.14(6) ° V = 1627(1) Å^3; μ(Mo-K_α) = 6.11 cm^{-1}; F(000) = 730.0; space group $P\bar{1}$ with Z = 2 and $D_{calcd.}$ = 1.41 g cm^{-3}. A selected specimen (0.56 × 0.25 × 0.19 mm.) was sealed in a thin-walled glass capillary. The unit cell parameters were derived from a least-squares refinement of 25 well-centered reflections by use of a Rigaku AFC-7R, and all diffractions were measured on a Rigaku R-AXIS-IV imaging plate area detector with graphite monochromated Mo-K_α radiation (λ = 0.71070 Å). A total of 5484 reflections with a maximum 2θ value of 51.4° were collected. The reflection data were corrected for Lorentz and polarization effects. No absorption correction was applied because of its small absorption coefficient.

The structure was solved by a combination of direct methods and Fourier techniques and refined anisotropically for non-hydrogen atoms by full-matrix least-squares calculations. Refinements were continued until all shifts were smaller than one-third of the standard deviations of the parameters involved. Atomic scattering factors and anomalous dispersion terms were taken from the literature.[18] Hydrogen atoms, which were located from the difference Fourier maps, were included for the structure factor calculation, but not refined. The final cycle of full-matrix least-squares refinement was based on 4725 observed reflections [$I > 3\sigma(I)$] and 388 variable parameters; final R and R_w values converged to 0.091 and 0.141, respectively, with the largest parameter being 0.04 times its esd. The standard deviation of an observation of unit weight was 1.28. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.75 and −1.24 e/Å^3, respectively, which were detected near the metal ion. All calculations were performed using the teXan[19] crystallographic software package from the Molecular Structure Corporation.

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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 threefold axis. The idealized twist angle is calculated by the following equation: twist angle = −73.9 + 94.1b, where b is a normalized bite and calculated from the bite angle, θ(O−Fe−O) = 2 *sin* (θ/2). See also refs. 2 and 9.
[11] [11a] BCT and BCTPT are the abbreviations for bicapped TRENCAM and bicapped TPTCAM, respectively, which are composed of three 2,3-dihydroxyterephthalic acids and two tris-

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[12] $\beta_{mlh} = [M_mL_lH_h]/[M]_m[L]_l[H]_h$ is defined as the overall formation constant for $M_mL_lH_h$ where M = metal ion, L = ligand in its deprotonated form, and H = hydrogen ion. The pK_as of TAGP ($pK_a^1 = 9.49$, $pK_a^2 = 9.18$, $pK_a^3 = 8.40$, $pK_a^4 = 7.61$) and the β_{111} of Fe^{III}-TAGP ($\log\beta_{111} = 30.6$) complex were determined by the potentiometric titration and the spectrophotometric measurement of competition reaction with EDTA according to ref. 2.

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[14] The siderophore-free ATCC No. 424 broth was prepared by the following method; 1 g Bacto Peptone, 1 g Yeast Extract and 0.2 g K_2HPO_4 were dissolved in 100 mL of water, and then the solution was adjusted pH to 7.4 with 1 M NaOH.

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[17] Crystallographic data (excluding structure factors) for the structure reported in this paper has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-161835. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [Fax: (internat.) +44-1223/336-033; E-mail: deposit@ccdc.cam.ac.uk].

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