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Corynebactin and a Serine Trilactone Based Analogue: Chirality and Molecular Modeling of Ferric Complexes

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Because the hydrolysis of ferric ion makes it very insoluble in aerobic, near neutral pH environments, most species of bacteria produce siderophores to acquire iron, an essential nutrient. The chirality of the ferric siderophore complex plays an important role in cell recognition, uptake, and utilization. Corynebactin, isolated from Gram-positive bacteria, is structurally similar to enterobactin, a well-known siderophore first isolated from Gram-negative bacteria, but contains L-threonine instead of L-serine in the trilactone backbone. Corynebactin also contains a glycine spacer unit in each of the chelating arms. A hybrid analogue (serine-corynebactin) has been prepared which has the trilactone ring of enterobactin and the glycine spacer of corynebactin. The chirality and relative conformational stability of the three ferric complexes of enterobactin, corynebactin, and the hybrid have been investigated by molecular modeling (including MM3 and pBP86/DN* density functional theory calculations) and circular dichroism spectra. While enterobactin forms a Δ-ferric complex, corynebactin is Λ. The hybrid serine-corynebactin forms a nearly racemic mixture, with the Λ-conformer in slight excess. Each ferric complex has four possible isomers depending on the metal chirality and the conformation of the trilactone ring. For corynebactin, the energy difference between the two possible Λ conformations is 2.3 kcal/mol. In contrast, only 1.5 kcal/mol separates the inverted Λ- and normal Δ-configuration for serine-corynebactin. The small energy difference of the two lowest energy configurations is the likely cause for the racemic mixture found in the CD spectra. Both the addition of a glycine spacer and methylation of the trilactone ring (serine to threonine) favor the Λ-conformation. These structural changes suffice to change the chirality from all Δ (enterobactin) to all Λ (corynebactin). The single change (glycine spacer) of the hybrid ferric serine-corynebactin gives a mixture of Δ and Λ, with the Λ in slight excess.

Introduction

The importance of siderophores for the acquisition of iron in microbes and the resulting bacterial pathogenicity is well established.[1] Microorganisms use these low molecular weight compounds to overcome the insolubility of Fe^{3+} at pH 7 ($\sim 10^{-18}$ M).[2] Specific receptor proteins on the cell membrane recognize the ferric complexes. After entering the bacterial cell, iron is released via reduction, hydrolysis, or ligand exchange mechanisms.[3] The siderophore with the highest known affinity for binding Fe^{3+} is enterobactin (**1**, Figure 1),[4] produced by both some Gram-positive and several Gram-negative bacteria like *E. coli*. Enterobactin has a high stability ($K_f = 10^{49}$),[5] with metal coordination at neutral pH through the six catecholate oxygens.[6] This coordination leads to a chiral iron center, which is Δ[6] in enterobactin. This chirality

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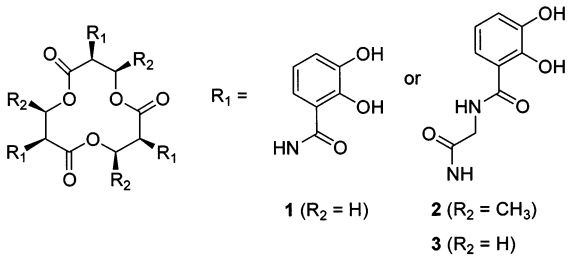

Figure 1. The siderophores enterobactin (**1**) and corynebactin (**2**). The synthetic analogue **3** is a hybrid, composed of the serine trilactone connected to the side chain of corynebactin.

is not essential for receptor recognition and transport through the membrane,[7] but it is necessary for the iron utilization inside the microbial cell. The mirror image, ferric-enantio-enterobactin complex, does not promote microbial growth,[8] indicating the importance of chirality for iron uptake.

A related siderophore, corynebactin (**2**), was described recently.[9] Two Gram-positive bacteria, *Corynebacterium glutamicum* and *Bacillus subtilus*, produce corynebactin, the second example of a siderophore with a trilactone backbone. Corynebactin consists of L-threonine units in contrast to enterobactin, where L-serine units are incorporated. Each corynebactin side chain also contains one glycine spacer. Recently, we described that the iron complexes of these two closely related siderophores have opposite chirality.[10,11] Additionally, we prepared serine-corynebactin hybrid compound **3** with a serine trilactone backbone and the corynebactin side chains which has properties similar to both enterobactin and corynebactin.[10,11] Herein, we report the circular dichroism spectra of Fe(III) complexes with **1**−**3** and establish the observed chiralities to be fully consistent with the results of conformational analyses performed with a molecular mechanics model.

Results and Discussion

Remarkably, the ferric complexes of compounds **1**−**3** all show different circular dichroism spectra. After the ferric complexes were prepared from the solutions of the free ligands in buffered water (pH = 7) with equivalent amounts of iron trichloride and purified with HPLC,[12] circular dichroism measurements were obtained.[13] In contrast to the Δ-iron(III)-enterobactin complex, the ferric corynebactin complex has a Λ-conformation. Ferric serine hybrid analogue **3** appears to be a mixture of Δ- and Λ-isomers, with a slight excess of Λ.

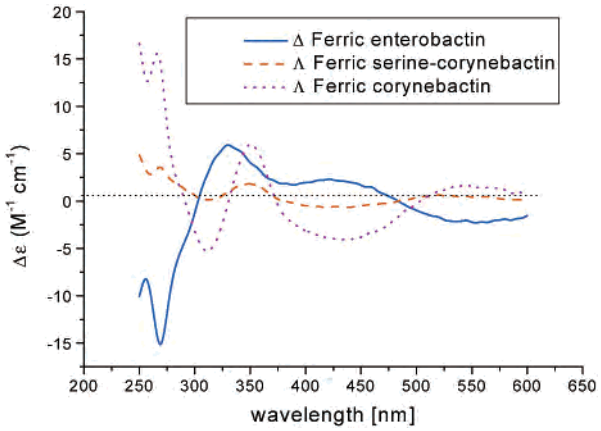

Figure 2. Circular dichroism spectra of ferric enterobactin, ferric corynebactin, and ferric serine-corynebactin in water. $T = 22$ °C.

Table 1. Circular Dichroism Results of Ferric Complexes

ferric complex	diastereomer	λ_{max} [nm]	$\Delta\epsilon$ [M^{-1} cm^{-1}]
enterobactin (**1**)	Δ	553	−2.2
corynebactin (**2**)	Λ	545	+1.7
serine-corynebactin (**3**)	Λ, slightly	520	+0.6

All ferric complexes of **1**−**3** reveal intense CD bands at 270 nm corresponding to the carbonyl amide in the ligand (Figure 2, Table 1). The bands of ferric corynebactin and serine-corynebactin (350 nm) and ferric enterobactin (330 nm) are due to the chiral trilactone scaffold. Two characteristic ferric catechol transitions are observed in the visible region at 435 nm and between 520 and 540 nm. These bands arise from ligand-to-metal charge transfer (LMCT) transitions and are therefore sensitive to the chirality at the metal center.[14]

To confirm these results, molecular modeling of the ferric complexes was carried out. MM3 calculations with an extended parameter set were used to perform a conformational search of the iron(III)-corynebactin complex.[15,16] The search identified three low lying conformations with C_3 symmetry (Figure 3, Table 2). A higher energy C_3 symmetry structure was also located. Interestingly, two of the conformers showed an inverted macrocycle with the carbonyl groups

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(12) Ligands (**1**−**3**) were dissolved in a mixture of 2 mL of water and 3 mL of methanol. The initial concentration of the complex was 0.5 mM. Ferric ion dissolved in 10 mM HCl was added to the solution to make a 1:1 complex. A color change was observed, and the solution was centrifuged for 10 min (14000 rpm eppendorf) to remove solid precipitate. Impurities were removed by preparative HPLC eluting with H_2O/MeOH (35:65), with a pressure of approximately 1000 psi and a flow rate of 10 mL/min. The intensity of the eluent was measured at 254 nm. The colored fraction of each ligand was collected.
(13) The pure fraction collected from HPLC was measured by UV−vis spectrophotometry (Cary 300 Scan). The concentrations of the ferric compounds were approximately 0.066 mM ($\epsilon = 15000$ M^{-1} cm^{-1} at 330 nm). The CD spectra of the complexes were measured using a Jasco J-810 spectrometer.
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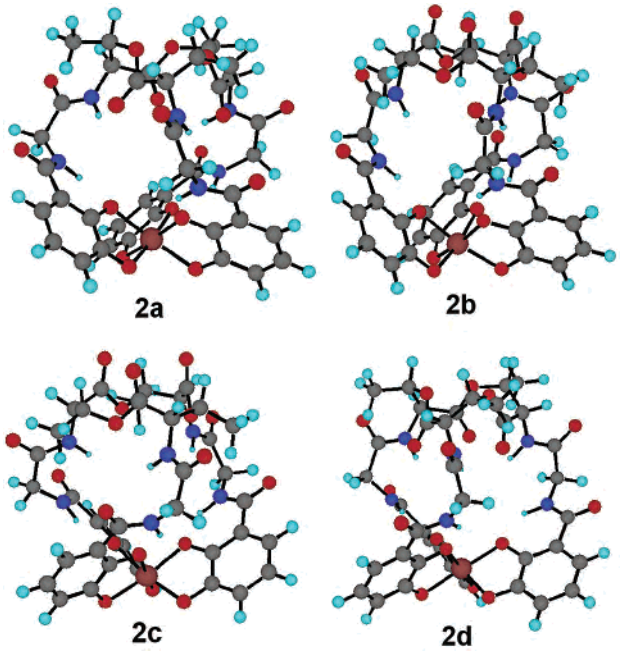

Figure 3. C_3 symmetry structures located by conformational searches of Fe^{3+} complexes with corynebactin (**2**).

Table 2. Conformations and Energies of the Ferric Complexes **2a**−**d**

ferric complex	diastereomer	energy [kcal/mol]	macrocycle
2a	Λ	0.0	inverted
2b	Λ	2.3	normal
2c	Δ	3.6	normal
2d	Δ	11.3	inverted

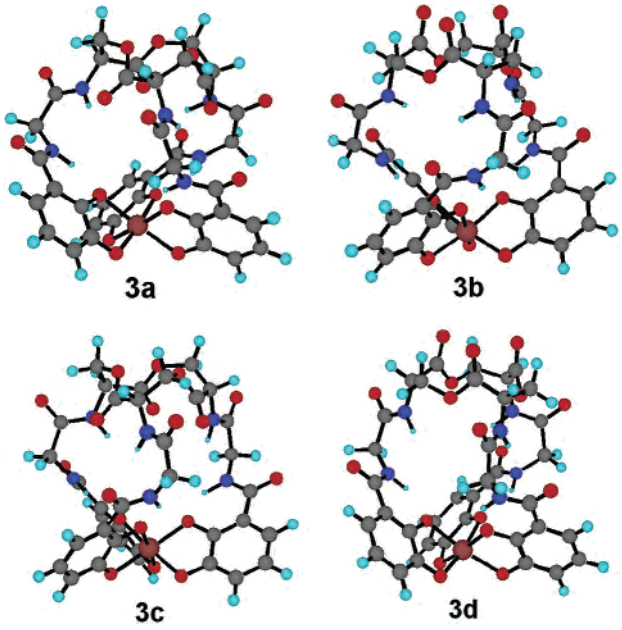

Figure 4. C_3 symmetry sturctures located by conformational searches of Fe^{3+} complexes with serine corynebactin (**3**).

pointing toward the metal ion. For both stereoisomers, the conformer with the inverted backbone has a lower energy than the "normal" one with the CO groups pointing outside. This "normal" structure was already known from the vanadium(IV)-enterobactin complex.[6] Both Λ-conformers **2a** and **2b** have the lowest energy of all four structures of **2**. The Δ-isomers **2c** and **2d** are higher in energy and therefore

Table 3. Conformations and Energies of the Ferric Complexes **3a**−**d**

ferric complex	diastereomer	energy [kcal/mol]	macrocycle
3a	Λ	0.0	inverted
3b	Δ	1.5	normal
3c	Δ	3.9	inverted
3d	Λ	5.1	normal

Table 4. Conformations and Energies of the Corynebactin Trilactone Macrocycle without Side Chains

trilactone structure	method	energy [kcal/mol]
normal	MM3	0.0
inverted	MM3	2.3
normal	pBP86/DN*	0.0
inverted	pBP86/DN*	3.2

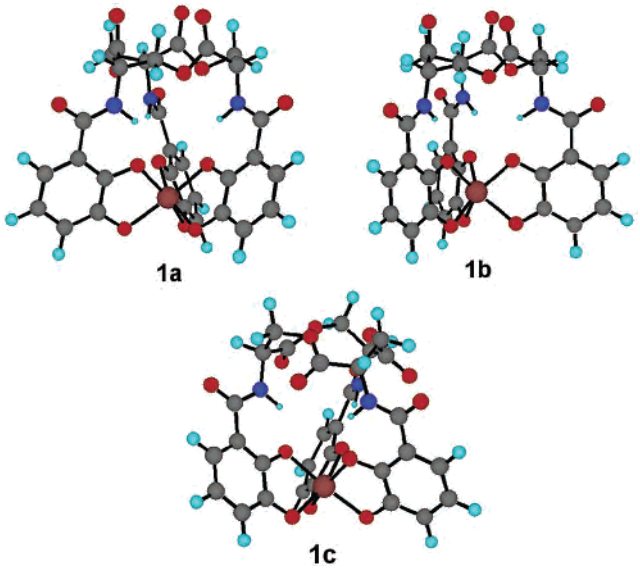

Figure 5. C_3 symmetry structures located for Fe^{3+} complexes with enterobactin (**1**).

Table 5. Conformations and Energies of the Ferric Complexes **1a**−**c**

ferric complex	diastereomer	energy [kcal/mol]	macrocycle
1a	Δ	0.0	normal
1b	Λ	2.1	normal
1c	Λ	2.2	inverted

represent smaller populations in solution. The CD spectrum of ferric **2** confirms this result showing an intense band for the ferric Λ-corynebactin complex (**2**).

These structures were then re-optimized with MM3 after removal of the methyl groups to yield the analogous C_3 symmetry structures **3a**−**d** for the serine corynebactin-Fe-(III) complex. The iron(III)-serine-corynebactin complex also has four stable conformers (Figure 4, Table 3). Similar to **2a**, the lowest energy conformer, **3a**, contains an inverted trilactone ring. In contrast to the conformers of corynebactin, **3b** (+1.5 kcal/mol) is Δ and contains the normal trilactone ring. The next isomer, **3c** (+3.9 kcal/mol), is also Δ, but contains the inverted trilactone backbone. The highest energy conformer (+5.1 kcal/mol) is again Λ, but contains the normal trilactone ring. Removal of the methyl groups appears to dramatically destabilize the Λ conformer with the normal trilactone ring. In this case, the energy difference between the lowest lying Δ form is not as large as it is for corynebactin. This smaller energy difference is consistent

Table 6. Calculated Structural Features of Lowest Energy Structures for the Ferric Complexes of **1**, **2**, and **3**[a]

complex	Fe—O_{ortho}	Fe—O_{meta}	O—Fe—O	Fe—O—C	Fe—O—C—C	N—H···O=C	N—H···O_{ortho}
1a	2.016	2.022	80.4	112.6	5.2	na	1.77
1b	2.033	2.039	79.0	113.7	2.5	na	1.79
1c	2.014	2.021	80.4	112.4	7.0	na	1.75
2a	2.012	2.016	81.7	111.6	4.9	2.00	1.82
2b	2.010	2.017	81.3	111.5	8.7	na	1.76
2c	2.011	2.019	81.4	111.2	6.6	na	1.78
2d	2.015	2.014	81.8	111.3	−1.0	2.16	1.83
3a	2.012	2.017	81.6	111.6	4.9	2.01	1.80
3b	2.010	2.019	81.3	111.7	6.3	na	1.77
3c	2.012	2.013	81.8	111.4	1.1	2.35	1.82
3d	2.009	2.015	81.5	111.5	7.5	na	1.77

[a] Units: distances in angstroms, angles in degrees. Ortho and meta designations refer to the catecholate oxygen atom position with respect to the amide substituent. The O—Fe—O value refers to the intrachelate angles only.

with a mixture of the Δ and Λ chiralities, with only a slight excess of Λ, as visible in the CD spectrum.

Further calculations were done to evaluate the relative stability of the normal and inverted forms of the corynebactin macrocyclic ring. The side chains were replaced by hydrogen atoms ($R_1 = R_2 = H$). The geometries were optimized with the molecular mechanics calculations using the MM3 program[15] and with density functional theory calculations (pBP86/DN*) using the MacSpartan program.[17] Without the side chain, the normal trilactone macrocycle is energetically favored by 2−3 kcal/mol (see Table 4). Perhaps the greater flexibility of the glycine containing arms allows for the inversion of the macrocycle to achieve a conformation recognized by Gram-positive bacteria. The effect of stereospecificity of the serine corynebactin analogue on receptor recognition is currently being investigated.

In prior work,[16] we reported Δ- and Λ-conformations for the iron(III)-enterobactin complex with the normal macrocycle. Here, we attempted to locate the inverted macrocycle forms for enterobactin. Either the Δ- or Λ-forms of the $[Fe(catecholate)_3]^{3-}$ complex were attached to the inverted triserine enterobactin backbone and then optimized with the MM3 model (Figure 5, Table 5). This approach yielded an inverted Λ-form. However, repeated attempts to locate an inverted Δ-form were unsuccessful.

Additional hydrogen bonds formed from the inversion of the trilactone are seen in the calculated structures (see Table 6). All normal and inverted structures contain hydrogen bonds between the three amides and the *ortho* oxygen atoms of the catechol moieties. However, the inverted structures of **2** and **3** also contain hydrogen bonds between the second set of amides attached to the trilactone ring and the inverted CO moieties. This additional stabilization may compensate for the unfavorable inversion of the trilactone ring. Because enterobactin only contains one set of amide hydrogens and these are involved in the hydrogen bonding with the *ortho* oxygen of the catechol, inversion of the CO moieties is disfavored because no stabilizing hydrogen bonds can be formed.

(17) The MacSpartan Program is available from Wavefunction, Inc., 18401 Von Karman Ave., Suite 370, Irvine, CA 92612.

Summary

The CD spectra of ferric complexes of enterobactin (**1**), corynebactin (**2**), and the serine-based analogue (**3**) suggest differing chiralities at the metal centers. This behavior has been confirmed by conformational analysis using an extended MM3 model.[16] In full agreement with experiment, the modeling results predict Δ chirality for **1**, Λ chirality for **2**, and, upon removal of the methyl substituents from the macrocycle, a decreased energy gap between the Δ and Λ forms of **3**. The calculations also reveal that the lowest energy forms of **2** and **3** have an inverted macrocycle conformation. Calculations on the isolated macrocycle show that, in the absence of side chains, the inverted conformer is only 2−3 kcal/mol higher in energy than the normal conformer. The inverted macrocycle conformer seen in **2** and **3** appears to be stabilized by hydrogen bonding between the macrocyclic CO groups and amide N—H hydrogen bond donors in the side chains.

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Supporting Information Available: Cartesian coordinates for the calculated compounds **1a**−**c**, **2a**−**d**, and **3a**−**d**. This material is available free of charge via the Internet at http://pubs.acs.org.

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