The Complex Structure of Ferri-ferribactins
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Z. Naturforsch. 55c, 836–839 (2000); received June 20, 2000
Pseudomonas chlororaphis, Pseudomonas fluorescens, Ferribactin
By comparison of the NMR data of the ferribactins from Pseudomonas chlororaphis ATCC 9446 and of P. fluorescens 18.1 with those of their Ga3+-complexes it will be shown that only two bidentate ligands are provided for complexation, both located in the peptide chain. The two remaining free sites of the octahedral metal ion are probably occupied by solvent molecules.

Introduction
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Orn-L-Ser-D-Ser-Gly-[L-Lys-L-Ser-FoOH-d-Orn)] were isolated and characterized as described earlier (Hohlneicher et al., 1992; Amann et al., 2000).

While Ga$^{3+}$-complexes of pyoverdins can readily be purified by chromatography (e.g., Amann et al., 2000), those of ferribactins decompose when a purification is attempted. Even when an excess of Ga(NO$_3$)$_3$ is added to a phosphate buffered solution of the ferribactin the complex formation is by far not complete as can be seen from the $^1$H-NMR spectra. The phosphate ions seem to compete as complexing agents. For NMR analyses the following procedure proved to be satisfactory: To 15 mg 2 dissolved in 3 ml H$_2$O 1.1 equivalents of Ga(NO$_3$)$_3$ dissolved in 1 ml H$_2$O were added drop by drop under stirring. A pH of 4.2 was determined potentiometrically after complete addition of the Ga salt. After 1 hr the sample was brought to dryness i.v. 15 mg of Ga-2 were dissolved in 0.9 ml H$_2$O and the pH was adjusted to 4.0 (potentiometric control); 0.1 ml D$_2$O were added for the lock signal.

**Results and Discussion**

Ga$^{3+}$-complexes have been used in several instances as models for Fe$^{3+}$-complexes which are not amenable to NMR spectroscopy, primarily to get information from the $^1$H-data about the three-dimensional structures in solution (e.g., Mohn et al., 1994). Both metal ions form octahedral complexes and the ion radius of Ga$^{3+}$ (62 pm) is very close to that of Fe$^{3+}$ (65 pm). Ga$^{3+}$ causes changes

<table>
<thead>
<tr>
<th>Pyoverdin</th>
<th>C-7</th>
<th>C-8</th>
<th>C-9</th>
<th>C-10</th>
<th>Fo</th>
<th>OhOrn</th>
<th>cyclo</th>
<th>Lit.</th>
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<tr>
<td>Pa 27853</td>
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<td>8.1</td>
<td>10.2</td>
<td>-4.1</td>
<td>-5.2</td>
<td>-6.7</td>
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<tr>
<td>Pf 51W</td>
<td>-5.1</td>
<td>9.2</td>
<td>11.0</td>
<td>-0.5</td>
<td>-6.1</td>
<td>-4.5</td>
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<td>2</td>
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<tr>
<td>Pf 18.1</td>
<td>-3.6</td>
<td>7.2</td>
<td>9.7</td>
<td>-3.9</td>
<td>-6.8</td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Pf PL7</td>
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<td>10.5</td>
<td>-4.3</td>
<td>-10.2</td>
<td>-6.9</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Pf PL8</td>
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<td>10.4</td>
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<td>-10.5</td>
<td>-7.0</td>
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<table>
<thead>
<tr>
<th>Ferribactin</th>
<th>C-3</th>
<th>Dab/Tyr (2, 3)</th>
<th>C-4</th>
<th>C-5</th>
<th>Glu</th>
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<tr>
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<td>0.0</td>
<td>0.1</td>
<td>0.1</td>
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<tr>
<td>3</td>
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<td>0.0</td>
<td>-0.1</td>
<td>0.2</td>
<td>-6.3</td>
<td></td>
<td></td>
<td>5</td>
</tr>
</tbody>
</table>

1 Tappe (1995); 2 Amann et al. (2000); 3 Voss et al. (1999); 4 Barelmann (1998); 5 present publication.
in the electron density at its binding sites resulting in chemical shift differences with reference to the free siderophore. This effect is especially notable for $^{13}$C-resonances. In the $^1$H-spectra influences due to conformational changes prevail, which bring certain structural units into different shielding or deshielding regions of the molecule. In Table I the shift differences for the binding sites of free pyoverdins and their Ga-complexes are compiled and compared with those of the two ferribactins. Note especially the effect on the C-atoms carrying the OH-groups of the catecholate system (C-8 and C-9) extending even to the neighboring C-atoms.

The shift differences observed for the formyl-CO of the ferribactins 2 and 3 (-6 ppm) agree with the values observed with pyoverdins. Hence, two ligands are provided by the two FoOHOrn units of 2 and 3. Clearly, the hydroxyl group of Tyr does not occupy one of the free complexation sites of Ga$^{3+}$: There are no shift differences observed for the 4-hydroxyphenyl ring of Tyr. Another candidate would have been the carboxyl group of the side chain Glu. However, the $\Delta$-values are negligible. This excludes a participation in the complex formation.

Structures and stoichiometries of dihydroxamate siderophore Fe$^{3+}$-complexes have been investigated in detail. Essentially two possibilities are under discussion, viz. the formation of 3:2-complexes with bridging ligands (e.g., Barclay et al., 1984) and of 1:1-complexes (monomeric or dimeric with the two ligands as bridges) where the free sites of the octahedral metal are occupied by solvent molecules (Caudle et al., 1994b). Equilibria may exist. In acidic media 1:1 complexes seem to prevail.

In the Ga complex of 2 several amino acids (Ser, Lys, Tyr and Glu) show doubled signals (shift differences <0.2 ppm) which might be interpreted as belonging to ferribactin ligands in a differing arrangement as in a 3:2-complex where two ligands are bound to one Ga$^{3+}$ each and the third ligand acts as a bridge between the metal ions. However, different conformations within an 1:1-complex had been observed also for pyoverdins and they resulted in an analogous doubling of signals. In favor of an 1:1-complex are the electrospray ionization mass spectral data. Between ca. pH 3 and 8 [2$^{+56}$Fe$^{3+}$-2H$^+$]$^+$ (m/z 1231.5 and [2$^{+56}$Fe$^{3+}$-H$^+$]$^2+$ (m/z 616.3) are formed. The isotope pattern of m/z 1231 shows that it is a singly charged species and not a doubly charged dimer (Caudle et al., 1994b). Solvent molecules occupying the remaining two ligand sites are lost readily in the electrospray process (Caudle et al., 1994a). However, after addition of 1,10-phenanthroline (phen) to the solution of Fe$^{3+}$-complex of 2 the ions [2$^{+56}$Fe$^{3+}$+phen]$^3+$ (m/z 471.4) and [2$^{+56}$Fe$^{3+}$+phen-H$^+$]$^2+$ (m/z 706.6) emerged. Phenanthroline is a more strongly bound ligand than H$_2$O or CH$_3$OH. The formation of the 1:1:1-complex confirms the assumption that only four ligand sites of Fe$^{3+}$ are occupied by the two bidentate FoOHOrn groups of 2, the remaining two being free for solvent molecules etc.


