The X-Ray Structure of the Pyochelin Fe^{3+} Complex

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By X-ray structure analysis it could be shown that from the solution equilibrium of pyochelin I and II, differing in the stereochemistry at C-2″ (**1a** and **1b**), crystals of the Fe^{3+} complex of the steroisomer **1a** are formed with a 1:1 metal-to-ligand ratio. Ligand sites are the carboxylate and the phenolate anions and the two nitrogen atoms. Two equivalent ferri-pyochelin moieties are held together by a hydroxy and an acetate unit which satisfy the remaining two coordination sites of Fe^{3+}.

Key words: Pyochelin, X-Ray Structure, *Pseudomonas aeruginosa*

Introduction

Pyochelin is one of the siderophores, *i.e.* iron sequestering metabolites (Budzikiewicz, 2004) of several bacterial species (Castignetti, 1997). It is described as an equilibrium mixture of two diastereomeric forms (Rinehart *et al.*, 1995; Zamri and Abdallah, 2000) differing in the configuration at C-2″ (Fig. 1): pyochelin I has the absolute configuration 4′*R*, 2″*R*, 4″*S* (**1a**), pyochelin II the configuration 4′*R*, 2″*S*, 4″*S* (**1b**) (Schlegel *et al.*, 2004). Two additional stereoisomers obtained during the synthesis (neopyochelin I and II) have a C-4′*S* configuration (Zamri and Abdallah, 2000; Zamri *et al.*, 2003).

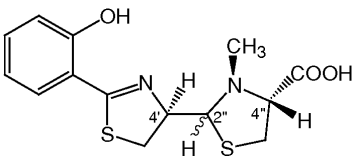

Fig. 1. Pyochelin stereoisomers (**1a**: 2″*R*; **1b**: 2″*S*).

Pyochelin forms a red Fe^{3+} complex. The stoichiometry pyochelin to Fe^{3+} was found by titration to be 2:1 at pH 2.5 (Visca *et al.*, 1992) in contrast to mass spectrometric studies which showed molecular ion species with a ratio 1:1 plus weak signals corresponding to a dimer (Cox *et al.* 1981; Beier and Stipanovic, 1989). The isolation of a 1:1:1 complex of Fe^{3+}, pyochelin and cepaciabactin

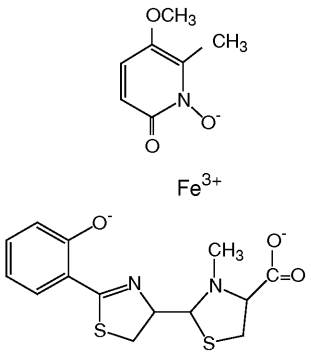

Fig. 2. Cepaciabactin-pyochelin Fe^{3+} complex.

(Klumpp *et al.*, 2005) suggests that four of the ligand sites of Fe^{3+} are satisfied by pyochelin while the remaining two bind cepaciabactin (Fig. 2). Cepaciabactin is a two-dentate ligand; three molecules bind one Fe^{3+} or Al^{3+} (Winkler *et al.*, 1986; Klumpp *et al.*, 2005). Recently an X-ray analysis of the ferri-pyochelin bound to the membrane receptor of *Pseudomonas aeruginosa* showed that one Fe^{3+} is complexed by pyochelin attached to the receptor. Pyochelin provides four of the binding sites, the remaining two are occupied by ethylene glycol stemming from the crystallization solution (Cobessi *et al.*, 2005).

None of the reported results indicate whether both, pyochelin I and II, can bind Fe^{3+}, and if not, which species is involved. X-Ray data will be presented here showing that from an aqueous solution a 2:2 complex of pyochelin I is obtained. Each pyochelin unit provides four binding sites (the car-

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boxylate and the phenolate anions plus the two nitrogen atoms of the heterocyclic rings). The two complex units are bridged by an acetate and a hydroxy ion.

Materials and Methods

Pyochelin Fe^{3+} complex

Pseudomonas aeruginosa PAO1 was grown in an artificial iron-free medium (Briskot *et al.* 1986). After removal of the cells the solution was brought to pH 1–2. Portions of 1 l supernatant were extracted three times with 250 ml acetic acid ethyl ester. After removal of the solvent *in vacuo* the residue was dissolved in 250 ml water and extracted three times with 200 ml acetic acid ethyl ester. The organic phase was washed with 100 ml water and the solvent was removed *in vacuo*. It remained an orange oil. To a solution of the oily residue from 1 l culture medium in methanol/glacial acetic acid 5:1 (v/v) 5 ml of a 5% solution of Fe(III) citrate in water were added and the mixture was concentrated *in vacuo* to 5 ml. After addition of 5 ml glacial acetic acid the solution was applied to a Biogel P-2 column equilibrated with a 0.2 M pyridinium acetate buffer (pH 5.0). Salts etc. were removed with the same buffer solution and the red Fe(III) complex was finally desorbed with a 2 M buffer and re-chromatographed under the same conditions. The solution was concentrated *in vacuo*, diluted with water/methanol and concentrated again several times to remove the buffer completely. 50 mg of the complex were dissolved in 4 ml methanol and applied to a polyamide column. Contaminants were removed with 300 ml methanol and 300 ml acetone/methanol/water 5:2:1 (v/v). Desorption was achieved with acetone/methanol/0.2 M acetic acid 5:2:1 (v/v). The solution was concentrated to one half of its volume and left over night while crystals separated.

X-ray analysis

All data were collected (Otwinowski and Minor, 1997) with a Nonius KappaCCD diffractometer (COLLECT, Nonius BV, Delft, NL, 1998). The structure (see Fig. 3) was solved using direct methods (SHELXS-97) followed by full-matrix least square refinement with anisotropic parameters for all non-hydrogen atoms, and isotropic parameters for H, using the riding model (SHELXL-97) (Sheldrick, 1997). Selected data of structure determination and refinement are presented in Table I. Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 299297. 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].

Results and Discussion

The X-ray structure analysis of the Fe^{3+} complex of pyochelin establishes a 1:1 ligand-to-metal ratio for the crystal structure with only one of the two diastereomers in equilibrium in solution. By de-

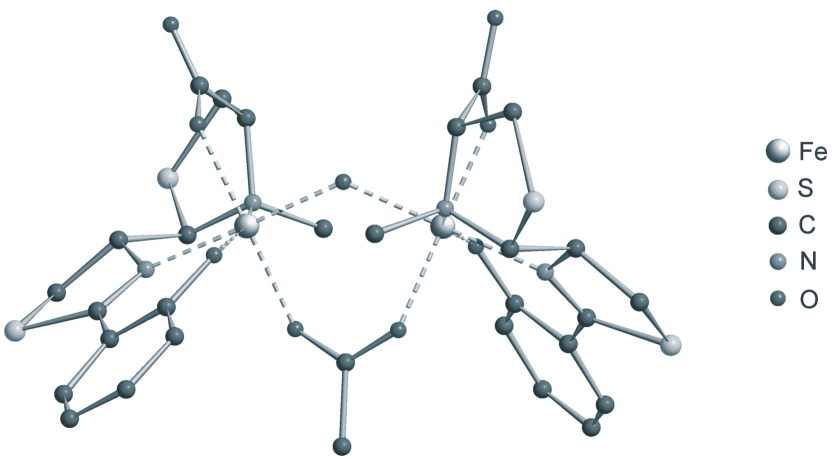

Fig. 3. Crystal structure of the pyochelin Fe^{3+} complex (without hydrogen atoms) [SCHAKAL99 (Keller, 1999)].

Table I. Crystal data and structure refinement for the pyochelin Fe^{3+} complex.

Identification code	pyochelin Fe^{3+} complex
empirical formula	$C_{30}H_{32}Fe_2N_4O_9S_4$
Formula weight	832.54
Temperature [K]	293(2)
Wavelength [Å]	0.71073
Crystal system, space group	monoclinic, *C*2
Unit cell dimensions	$a = 21.227(1)$Å $\alpha = 90°$ $b = 17.592(1)$Å $\beta = 132.47(1)°$ $c = 14.365(1)$Å $\gamma = 90°$
Volume	3956.8(4)
Z	4
Calculated density	1.398 g/cm^3
Absorption coefficient	0.995 mm^{-1}
F(000)	1712
Crystal size	$0.25 \times 0.25 \times 0.20$ mm^3
Θ Range for data collection	1.74° to 27.00°
Limiting indices	$-27 \leq h \leq 27$, $-22 \leq k \leq 22$, $-18 \leq l \leq 16$
Reflections collected / unique	16056 / 8402 [R(int) = 0.0210]
Completeness to theta = 27.00	99.2%
Absorption correction	none
Refinement method	full-matrix least-squares on F^2
Data / restraints / parameters	8402 / 1 / 474
Goodness-of-fit on F^2	1.056
Final *R* indices [$I > 2\sigma(I)$]	$R1 = 0.0523$, $wR2 = 0.1598$
R Indices (all data)	$R1 = 0.0580$, $wR2 = 0.1660$
Absolute structure parameter	0.006(19)
Largest diff. peak and hole	0.931 and −0.430 e/Å^3

complexation and subsequent NMR analysis it could be shown (Schlegel *et al.,* 2004) that the complexed form corresponds to pyochelin I (**1a**). Four of the six octahedral coordination sites of Fe^{3+} are occupied by the phenolate and the carboxylate oxygen and by the two nitrogen atoms of **1a**. The two remaining points accommodate the oxygen atom of a hydroxy and an acetate anion, the bridging units between the two equivalent ferri-pyochelin parts. In the crystal between the ferri-pyochelin units there are cavities which contain not ordered solvent molecules.

Apparently ferri-pyochelin can satisfy the two remaining octahedral positions of Fe^{3+} by any ligand that may be available, as the examples discussed above demonstrate. Pyochelin has a complexing constant of ca. 10^5, much lower than that of the peptidic pyoverdins, the main siderophores of *Pseudomonas* spp. (Budzikiewicz, 2004). The biosynthetically more economic pyochelin will be useful as a siderophore only when sufficient iron is available. This would be a strategy comparable to that of the related genus *Azotobacter* which produces catecholate siderophores at comparatively high iron concentrations, and the peptidic azotobactin (structurally related to the pyoverdins) only when the concentrations drop below a certain limit (Cornish and Pagem, 1995).

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