Diastereomeric Pyoverdin-Chromium(III) Complexes[†]

Herbert Budzikiewicz*, Halka Georgias and Kambiz Taraz

Institut für Organische Chemie der Universität zu Köln, Greinstr. 4, D-50939 Köln, Germany.
Fax: +49-221-470-5057. E-mail: aco88@uni-koeln.de

* Author for correspondence and reprint requests

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Coordination isomeric diastereomeric Cr^{3+} complexes of the pyoverdin of *Pseudomonas aeruginosa* ATCC 15692 could be separated by chromatography and characterized by spectroscopic methods.

Introduction

Pyoverdins are the siderophores, i.e. iron sequestering compounds, of the fluorescent group of the bacterial genus *Pseudomonas*. They consist of a dihdroxyquinoline chromophore and a peptide chain (Budzikiewicz, 1997). With Fe^{3+} they form octahedral complexes. The ligands are the catecholate grouping of the chromophore and hydroxamic acid and/or α-hydroxycarboxylic acid units located in the peptide chain. The ligands are non-symmetric and hence sixteen diastereomeric arrangements are theoretically possible, not all of which can actually be formed in a given pyoverdin complex for sterical reasons (Drechsel and Winkelmann, 1997). Octahedral d_5 high-spin Fe^{3+} complexes do not have ligand-field stabilization and are therefore kinetically labile. In solution a fast equilibrium between diastereomers could exist. There is no direct way to get information about such equilibria. An indirect approach is the replacement of Fe^{3+} by other trivalent metals which also form octahedral complexes. Most widely used is Ga^{3+} whose ionic radius (62 pm) is only 3 pm smaller than that of Fe^{3+}. Ga^{3+} is diamagnetic and thus allows NMR studies, which were used i.a. to calculate three-dimensional structures of two pyoverdin complexes (Mohn *et al.*, 1994; Atkinson *et al.*, 1998). Here and in other publications where NMR data of Ga^{3+} complexes are reported (Amann *et al.*, 2000, Voss *et al.*, 1999; Weber *et al.*, 2000) no indications for the presence of more than one diastereomeric form are mentioned, with the exception of the pyoverdin **1** of *Pseudomonas aeruginosa*, variously referred to as pyoverdin D (Briskot *et al.*, 1988), Pa A (Demange *et al.*, 1990) and PaO1 (Meyer *et al.*, 1997). Briskot (1988) mentions that from the two formyl signals of the N^4-formyl-N^4-hydroxy-Orn ligands only one is sharp while the other one (and also that of the neighboring CH_2-group of Orn) is broadened. This could indicate that two diastereomers may exist with different orientations of this hydroxamate ligand. In a short note NMR evidence is mentioned that in the analogous Al^{3+} complex two coordination isomers exist (Mertz *et al.*, 1991).

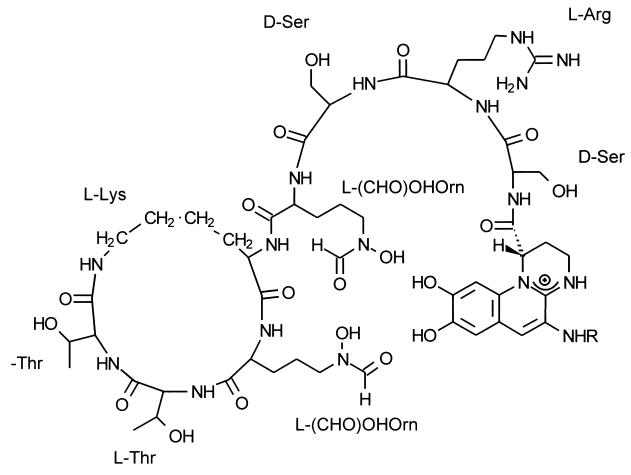
There is only one pyoverdin Fe^{3+} complex which could be obtained in crystalline form suitable for a X-ray analysis (Teintze *et al.*, 1981). In this context the possible existence of interconverting coordination isomers in solution is discussed which could be trapped as Cr^{3+} complexes. The octahedral d^3 complexes of Cr^{3+} shows a large ligand field stabilization and should be kinetically less labile than those of Fe^{3+}. A chromatographic separation could be possible. However, by TLC only one spot was obtained. In a recent publication Schalk *et al.* (2002) report that pyoverdin PaA forms with Cr^{3+} an equilibrium of slowly interconverting complexes, but no details are given as to how many it are and whether they differed in their CD spectra etc. We wish to report the synthesis, separation and characterization of two forms of the Cr^{3+} complex of pyoverdin D.

Materials and Methods

Analytical instruments

Mass spectrometry: H-SQ 30 (Finnigan-MAT, Bremen) with FAB gun (Ion Tech Ltd, Teddington, GB), FAB gas Xe, matrix thioglycerol/dithiodiethanol. The samples were freed from salts by adsorption on Sep-Pak RP-18 columns, rinsing with H_2O and subsequent elution with H_2O/

[†] Part CXI of the series "Bacterial constituents". For Part CX see Barelmann *et al.*, 2002b; for Part CIX Barelmann *et al.*, 2002a.



1. R = CO-CH_2-CH_2-COOH

Fig. 1.

CH_3OH 1:1 v/v. UV/Vis: Lambda (Perkin-Elmer, Überlingen). CD: Jasco J-40 S (Jasco, Tokio, Japan), solvent 25 mM phosphate buffer pH 7.0. Preparative HPLC: Wellchrom K 1000 Maxi Star (Knauer, Berlin), column polygosil 60 (C_{18}) 250 × 8 mm, solvent (a) 0.1 M CH_3COONH_4 in 1 l H_2O (pH 6.5) and (b) CH_3OH, gradient 5 to 30% (b) in 30 min. High tension paper electrophoresis: HVE System, 60,600 (Camad, Muttenz, CH), solvent 25 mM phosphate buffer, pH 7.0, desferral and glucose as references.

Synthesis and isolation of the Cr^{3+} complexes

Pyoverdin D from *Pseudomonas aeruginosa* ATCC 15692 was isolated and purified as described earlier (Briskot *et al.*, 1989). 20 mg of pyoverdin D were dissolved in 7 ml dry dimethylsulfoxide containing 0.65 vol.-% triethylamine. With stirring under nitrogen 55 mg $CrCl_3$.3 (tetrahydrofuran) in 3 ml of the same solvent were added. The solution turned dark green and after 15 min the fluorescence of the pyoverdin had disappeared. Stirring was continued for 24 h, then 10 ml H_2O were added to the reaction mixture. The mixture was adsorbed on XAD-4 resin (30 × 2 cm) and washed with 200 ml H_2O to remove salts and solvents. The reaction products were eluted with H_2O/CH_3OH 1:1 v/v. The eluates were brought to dryness i.v. Yield 4.2 mg (21%). The mixture of complexes was separated by preparative HPLC (see above).

Results and Discussion

By preparative HPLC two fractions of about equal abundance were obtained (with increasing retention time I and II). In solution they show slow equilibration. After one week starting from either diastereomer a ratio of I:II of about 1:2 is reached. Both I and II showed $[M+H]^+$ ions in their FAB mass spectra at *m/z* 1383 corresponding to [pyoverdin-3 H + $^{52}Cr]H^+$. At pH 7.0 according to their electrophoretic mobility they are not charged. Both show an abundant absorption band (25 mM phosphate buffer, pH 7.0) at 411 nm (log ε 4.30) in agreement with the value reported for the pseudobactin Cr^{3+} complex (Teintze *et al.*, 1981), viz. 416 nm (log ε ÷4.30). It corresponds to the absorption of the chromophore. As can be seen from other octahedral Cr^{3+} complexes (Leong and Raymond, 1974a, 1974b and 1975; Isied *et al.*, 1976; Chung *et al.*, 1986) absorptions due to the spin-allowed d-d transitions expected at about 400 to 425 and at 580 to 600 nm are rather week and can not be detected, as it was also noted for the pseudobactin complex.

For ferri-pyoverdins the CD extrema at ca. 440 and 500 nm are assumed to be derived from a broad metal-to-ligand charge transfer band. As the hypsochromic one may be obscured by the CD band of the chromophore a positive Δε at ca 500 nm is correlated with a Λ configuration (Piper, 1961) of the complex. For Cr^{3+} pseudobactin CD bands at 412 (−4.6), 582 (+6.8) and 702 (−1.2) nm (Δε) are reported (Teintze *et al.*, 1981) in

agreement with other Λ-configurated octahedral Cr^{3+} complexes (ca. 420 to 460, 560 to 580 and 650 to 680 nm) (Leong and Raymond, 1974a, 1974b and 1975; Isied *et al.*, 1976; Chung *et al.*, 1986). They are brought into connection with the weak bathochromic d-d transitions.

For the Cr^{3+} complexes I and II only one CD band was detected (I: 440, −1.73; II +1.34). It lies in the region of the hypsochromic d-d absorption, but any interpretation (additional diastereomers hiding under the two HPLC fractions; just reversal of the orientation of one hydroxamic acid ligand as indicated above) could only be speculative. This investigation shows that the preparative separation of diastereomeric Cr^{3+} complexes of pyoverdins is possible and that in solution they are sufficiently stable for spectroscopic characterizations. Since more than fifty pyoverdin structures have been elucidated by now (Fuchs and Budzikiewicz, 2001) it would be a rewarding field of research to try to isolate and structurally identify additional Cr^{3+} complexes.

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