Structure proposal for a new pyoverdinin from a Thai *Pseudomonas putida* strain¹

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Abstract. From a Thai *Pseudomonas putida* strain a pyoverdinin was isolated whose primary structure was deduced from its mass spectrometric fragmentation pattern. It belongs to the smallest representatives of this group of siderophores comprising only six amino acids.

Keywords: *Pseudomonas putida*, pyoverdinin, siderophore

Abbreviations

Common amino acids, 3-letter code; Dab, 2,4-diaminobutanoic acid; AcOHOrn, N⁴-acetyl-N⁴-hydroxy Orn; Suc, succinic acid residue; Chr, pyoverdinin chromophore (see Fig. 1); MS, mass spectrometry; ESI, electrospray ionization; CA, collision activation.

1. Introduction

Pseudomonas putida is a member of the fluorescent species in the rRNA homology group I of the family Pseudomonadaceae which produces siderophores (“pyoverdins”) with high Fe³⁺ complexing constants. Today about 50 complete or fairly complete pyoverdinin structures have been elucidated and from preliminary studies it appears that many more are to be expected [1,2]. Pyoverdins consist of three distinct structural parts, viz. a dihydroxyquinoline chromophore responsible for their fluorescence, a peptide chain comprising 6 to 12 amino acids bound to the chromophore carboxyl group, and a small dicarboxylic acid (or its monoamide) connected amidically to its NH₂-group (cf. 1). The peptide chains have a twofold function. They provide two of the ligand sites for Fe³⁺, and they are responsible for the recognition of the ferri-pyoverdins by specific receptors located at the surface of the producing cell [3]. The variability of the peptide chain is closely connected with the second function: It safeguards that a given ferri-pyoverdinin is available only to the producing strain because of the usually highly specific interaction between the ferri-pyoverdinin and its receptor outer membrane protein [4].

Over the years much knowledge has been accumulated on the fragmentation behavior of pyoverdins after electrospray ionization (ESI) and collisional activation (CA) [1] which in many cases allows the

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determination of the amino acid sequence of the peptide chain even from crude bacterial culture extracts without previous isolation of the pyoverdin. We wish to demonstrate this for a pyoverdin obtained from a Thai *Pseudomonas putida* strain.

2. Materials and methods

Mass spectrometry: Finnigan-MAT 900 ST with an ESI source, solvent CH₃OH/H₂O 1 : 1. Mass selected fragmentation by CA was conducted either in the quadrupole region in front of or in the ion trap. In the text nominal masses are reported, Figs 2–4 give the exact masses.

The bacterial strain was obtained from the Science and Technology Research Institute of Thailand, Bangkok and identified phenotypically as a *Pseudomonas putida*. It was grown in a succinate minimal medium. For the work-up of the culture after addition of ferric citrate and the isolation of the ferri-pyoverdins by chromatography on XAD-4 resin see [5].

3. Results and discussion

The molecular mass of **1** was determined by ESI-MS as 1016u. For structure elucidation CA of [M+H]⁺ and of [M+2H]²⁺ both in the quadrupole region and in the ion trap of the mass spectrometer gave complementary results. In the [M+H]⁺ ions the proton is located in the chromophore as depicted in Fig. 1. Fragment formation in the peptide chain occurs by charge-remote processes. This results in a limited number of characteristic ions. In the [M+2H]²⁺ ions the second proton can be located anywhere in the peptide chain and foster cleavages there. A larger number of structure relevant fragment ions will result, but abundant doubly charged ions can obscure parts of the spectrum. In the quadrupole region several collision induced activations may occur and ions resulting from consecutive fragmentation will then be observed. By CA in the ion trap of a selected ion only single-step fragmentation processes are possible, but the relatively long lived ions in the trap may partially undergo rearrangement processes.

In the quadrupole CA spectrum of [M+2H]²⁺ (Fig. 2) (*m/z* 509) the *retro*-Diels-Alder fragmentation of the chromophore [6] can be observed. Loss of C-2 and C-1 of the chromophore together with the peptide chain and subsequent elimination of the succinic acid side chain gives an ion at *m/z* 204 characteristic for a pyoverdin chromophore (in contrast to isopyoverdins). Ions at *m/z* 86, 114 and 131 are

Fig. 1. Structure proposed for pyoverdin **1**.

Fig. 2. Quadrupole CA spectrum of $[M+2H]^{2+}$ (m/z 509.3) (for the ions characteristic for Orn, m/z 86–131, and for the chromophore, m/z 204, see text).

Scheme 1. Formation of B-ions (R^1 and R^2 are the amino acid side chains).

Scheme 2. Formation of the ion m/z 260 (**a**) from **1**.

in agreement with the presence of a N^4 -acyl- N^4 -hydroxy-ornithine residue. Most pronounced is a fragment ion at m/z 260 (**a**) to be found in the quadrupole CA spectra of $[M+H]^+$ and of $[M+2H]^{2+}$, but not in the ion-trap CA spectra. This is indicative of a multi-step decomposition. Its precursor is probably an ion at m/z 360 which can lose H_2O (m/z 342) and the succinic acid residue leaving the free C-5 amino group, a frequently observed fragmentation process (cf. the formation of m/z 204 above). A similar pattern was observed for the pyoverdins **R'** [7] (unpublished mass spectrometric data). It seems to be characteristic for pyoverdins where the condensation product of Ser and Dab follows immediately the chromophore. Its formation does not follow the typical B-ion genesis with protonation at the amide nitrogen (Scheme 1), but it is rather due to protonation of the chromophore and transfer of one hydrogen (Scheme 2). The presence of this condensation product is confirmed by the loss of CH_2O

Scheme 3. Loss of CH_2O from the Dab/Ser condensation product. Figure 3. Ion trap CA spectrum of $[\text{M}+2\text{H}]^{2+}$ (m/z 509.3) (B ions show loss of H_2O , -18 u, of CH_2O , -30 u, and of $\text{CH}_2\text{O} + \text{H}_2\text{O}$, -48 u; see text).

from the protonated molecular and from several fragment ions (Scheme 3) [8]. Ions resulting from the cleavage of the peptide chain are B_0 (m/z 358) and $[\text{B}'_5]^{2+}$ (m/z 444).

The ion trap CA spectrum (MS^2) of $[\text{M}+2\text{H}]^{2+}$ (Fig. 3) allows to deduce the amino acid sequence of **1** from the B-fragment series (cleavage of the amide bonds with charge retention at the N-terminal fragment; [9]) (Table 1). All B-ions show in addition losses of H_2O and of CH_2O (see above). Of high abundance are the ions $[\text{M}+2\text{H}-\text{H}_2\text{O}]^{2+}$ (m/z 500) and $[\text{B}'_5]^{2+}$ (m/z 444).

In the molecular ion region of the quadrupole CA spectrum of $[\text{M}+\text{H}]^+$ the losses of H_2O and of CH_2O can be seen. In the lower mass region occur ions also observed in the quadrupole CA spectrum of $[\text{M}+2\text{H}]^{2+}$. More informative is the ion trap CA spectrum (MS^2) of $[\text{M}+\text{H}]^+$ (Fig. 4). Thus, the Y''_6 ion (the protonated complete peptide chain) at m/z 660 and $[\text{Y}''-\text{H}_2\text{O}]^+$ becomes extant. In addition, two rearrangement ions are observed, viz. $[\text{B}_2+\text{H}_2\text{O}]^+$ (m/z 545) resulting from an OH-transfer from serine, and $[\text{B}_3+42]^+$ (m/z 670) involving an acetyl migration from AcOHOrn to Dab [8]. As to be expected in the ion trap CA spectrum of the ion m/z 987 ($[\text{M}+\text{H}-\text{CH}_2\text{O}]^+$) the ions B_2 – B_4 and Y''_6 as well as $[\text{B}_2+\text{H}_2\text{O}]^+$ are also shifted by 30 u to lower masses.

Table 1
B-ions^a in the MS-CA spectrum of $[M+2H]^{2+}$ of **1**

<i>n</i>		B	-H ₂ O	-CH ₂ O
0	Suc-Chr	358		
1/2	Ser/Dab	527	509	497
3	Thr	628	610	598
4	Ser	715	697	685
5	AcOHOrn	887	869	857

^aDoubly charged ions B₄ and B₅ are observed.

Figure 4. Ion trap CA spectrum (characteristic part) of $[M+H]^+$ (m/z 1017.5).

The results show that it is possible to propose the structure **1** (Fig. 1) of a pyoverdin (except the chirality of the amino acids present in its peptide chain) from the analysis of its mass spectrometric fragmentation patterns after CA of the $[M+H]^+$ and $[M+2H]^{2+}$ ions obtained by ESI-MS. It is important to get both the quadrupole and ion trap CA spectra as they give complementary information.

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