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Metal trafficking via siderophores in Gram-negative bacteria: Specificities and characteristics of the pyoverdine pathway

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Abstract

Under iron-limiting conditions, fluorescent pseudomonads secrete fluorescent siderophores called pyoverdines (Pvd), which form complexes with iron that are then taken up by the bacteria. Pvds consist of a fluorescent chromophore derived from 2,3-diamino-6,7-dihydroxyquinoline and containing one of the bidentate groups involved in iron chelation, linked to a peptide moiety containing the other two bidentate groups required for binding to Fe^{3+}. More than 100 different Pvds have been identified, with different peptide sequences, forming a wide family of siderophores. In the human opportunistic pathogen *Pseudomonas aeruginosa*, Pvd is necessary for infection and is considered to be a virulence factor. This review focuses on the mechanisms underlying iron uptake by the Pvd pathway in pseudomonads, taking into account recent biochemical and biophysical studies and recently solved 3D-structures of the Pvd outer membrane transporter FpvA in four different loading states. These data are discussed and compared with the mechanisms of siderophore–Fe uptake reported for other Gram-negative bacteria.

Keywords: Siderophore; Iron uptake; Pyoverdine; Outer membrane transporter; TonB; *Pseudomonas*

1. Introduction

Iron is an essential element for the growth of most micro-organisms. It acts as a cofactor for the redox-dependent enzymes involved in most cellular processes, including electron transfer, RNA synthesis and resistance to reactive oxygen intermediates [1]. Under aerobic conditions, free iron abundance is limited by the very low solubility of ferric hydroxide. Bacteria and fungi have therefore developed efficient ferric ion-chelating agents, called siderophores, to scavenge iron from the extracellular environment and import it, making it possible to maintain adequate intracellular levels of iron [2–4]. *Pseudomonas,* a widespread bacterial genus, may be classified into five genetic homology groups [5]. One of these groups – fluorescent pseudomonads – is characterised by the production of yellow-green, water-soluble compounds called pyoverdins (Pvds) in iron-deficient conditions [6,7]. More than 100 different Pvds have been identified, forming a wide class of siderophores with a great diversity of structures. Not all of these molecules have been studied to the same extent. The Pvd produced by the human opportunistic pathogen *Pseudomonas aeruginosa* (PvdI, Fig. 1) is the archetype of this group of molecules, the pathogenicity of this bacterium ensuring its notoriety. *P. aeruginosa* infections are severe, and are frequently lethal in immunocompromised patients and patients with cystic fibrosis. During infections, this bacterium produces Pvd as a means of obtaining to iron, in conditions of strong competition with the host. This siderophore is therefore considered to be a virulence factor, essential for bacterial virulence [8].

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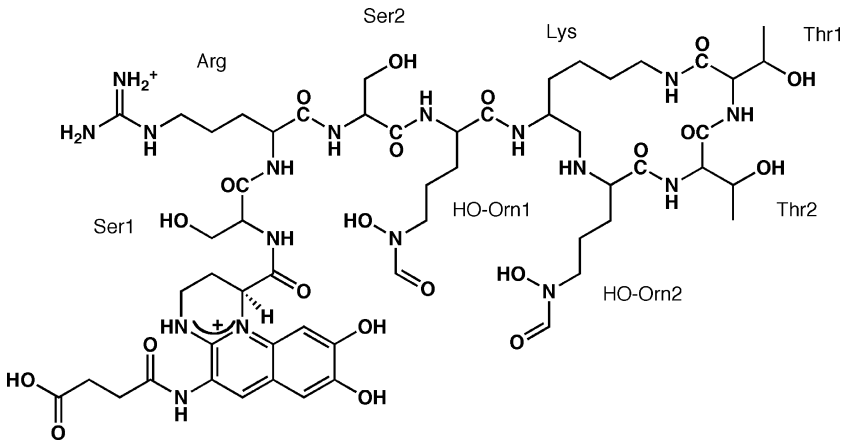

Fig. 1. The primary structure of PvdI, siderophore of *Pseudomonas aeruginosa* ATCC 15692.

The intrinsic fluorescence of Pvds has made it possible to obtain large amounts of data on this iron uptake system in *P. aeruginosa*. The spectral characteristics of iron-free Pvd or Pvd loaded with Ga and FpvA (outer membrane transporter of Pvd) are almost ideal for the observation of FRET (fluorescence resonance energy transfer) between Pvd and the Trp residues of its bacterial membrane transporter, when these two molecules are in close contact, such as during the binding of Pvd [9,10]. FRET is one of the most powerful techniques available for monitoring protein ligand interactions over time. These functional data obtained and the structures of FpvA in four loading states (FpvA, FpvA–Pvd, FpvA–Pvd–Fe and FpvA–Pvd–Ga [11–13]) indicate that this iron uptake pathway has certain features in common with other Gram-negative siderophore uptake pathways and haemophore trafficking, but that it also differs from these pathways. We review here the specific features and characteristics of the Pvd uptake pathway. The FpvAI transporter is also involved in a signalling cascade controlling the expression of *fpvA* and genes related to Pvd biosynthesis. This cascade involves a transmembrane signalling system induced by the binding of the siderophore to the outer membrane transporter, and the extracytoplasmic function (ECF) sigma factor/anti-sigma factor pairs, FpvI/FpvR and PvdS/FpvR [8,14–20]. This aspect of the PvdI uptake pathway will not be discussed here (for review see [18]).

2. Pyoverdine

All Pvds consist of a chromophore derived from 2,3-diamino-6,7-dihydroxyquinoline, with a peptide moiety bound to the chromophore and a side chain bound to the nitrogen atom at position C-3 of the chromophore. In most cases, this side chain is a diacid of the Krebs cycle, such as succinic, malic or α-ketoglutaric acid or one of their amide derivatives. The composition and length of the peptide are unique to each strain. Determinations of about 50 primary structures of Pvd have shown that the peptide moiety is usually composed of 6–12 amino acids and may be linear, or partially or entirely cyclic [6,21]. As the chromophore is common to all Pvds of fluorescent pseudomonads, the specificity with which the siderophore is recognised by its outer membrane transporter is attributed to the peptide region. In *P. aeruginosa*, three structurally different Pvds (with different peptide chains) have been identified (Pvd types I, II and III) [22–24]. Each strain of *P. aeruginosa* produces one of these three types of Pvd.

Pvds have an extremely high affinity for the ferric ion, with association constants as high as $10^{32}\,M^{-1}$ [25]. The chromophore forms the first bidentate group and the peptide moiety contains the other two bidentate chelating groups. Together, they chelate iron with 1:1 stoichiometry, resulting in complete and efficient iron(III) complexation. The second and the third bidentate group may be N^{δ}-acyl, N^{δ}-hydroxyornithine or β-hydroxyaspartic acid residues. The three-dimensional structures of only three Pvds have been resolved (Fig. 2): the crystal structure of pseudobactin A from *Pseudomonas* B10 and the NMR structure of gallium(III) complexes of Pvd GM-II and Pvd G4R from *Pseudomonas fluorescens* and *Pseudomonas putida* G4R, respectively [26–28]. Comparison of these three Pvd structures showed that co-ordination may differ between Pvds. Pseudobactin B10–Fe(III) and Pvd–Ga(III) have Λ configurations around the metal ion, whereas Pvd G4R–Ga(III) has a Δ configuration. The features common to the overall structure of these three Pvds are all located at one edge of the structure, defining a site of open access for each Pvd. However, both the orientation of this open access site with respect to the chromophore and the peptide fold differs in these three known structures. This lack of structural similarity, due to differences in the folding of the peptide moiety around the co-ordination sphere of the metal, accounts for the specificity of recognition and transport by receptors and strongly suggests that the determinants of this specificity are located in the peptide moiety and differ between Pvds.

The diversity in Pvd primary structures suggests that a large variety of enzymes may be required for the synthesis of these molecules. The peptides and chromophores of all