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Cellular organization of siderophore biosynthesis in *Pseudomonas aeruginosa:* Evidence for siderosomes

Véronique Gasser, Laurent Guillon, Olivier Cunrath, Isabelle.J. Schalk *

UMR 7242, Université de Strasbourg-CNRS, ESBS, Blvd Sébastien Brant, Illkirch, Strasbourg F-67413, France

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ABSTRACT

Pyoverdine I (PVDI) and pyochelin (PCH) are the two major siderophores produced by *Pseudomonas aeruginosa* PAO1 to import iron. The biochemistry of the biosynthesis of these two siderophores has been described in detail in the literature over recent years. PVDI assembly requires the coordinated action of seven cytoplasmic enzymes and is followed by a periplasmic maturation before secretion of the siderophore into the extracellular medium by the efflux system PvdRT-OpmQ. PCH biosynthesis also involves seven cytoplasmic enzymes but no periplasmic maturation. Recent findings indicate that the cytoplasmic enzymes involved in each of these two siderophore biosynthesis pathways can form siderophore-specific multi-enzymatic complexes called siderosomes associated with the inner leaflet of the cytoplasmic membrane. This organization may optimize the transfer of the siderophore precursors between the various participating enzymes and avoid the diffusion of siderophore precursors, able to chelate metals, throughout the cytoplasm. Here, we describe these recently published findings and discuss the existence of these siderosomes in *P. aeruginosa*.

1. Introduction

Siderophores [1] are secondary metabolites produced by bacteria in order to get access to iron, which is absolutely essential for bacterial growth. The ions of this metal are central to respiration processes and act as a cofactor for many enzymes involved in fundamental biological functions. However, iron bioavailability in the environment is low because ferric iron is poorly soluble under aerobic conditions and at physiological pH. Therefore, to acquire iron, bacteria have developed a strategy based on the secretion of small organic chelators called siderophores. The molecular masses of most of these compounds are between 200 and 2000 Da, and their chemical structures are very diverse [1,2]; nevertheless, they share the main characteristic of a very high affinity for iron, which allows them to solubilize iron in the bacterial environment. The ferri-complexes formed are then recognized at the bacterial surface and transported into the cells by specific transporters [3,4]. This strategy is used by microorganisms, in all bacterial environments, including the rhizosphere or in hosts during bacterial infection.

The opportunistic Gram-negative bacterium *Pseudomonas aeruginosa* secretes two major siderophores (Fig. 1): a fluorescent peptide siderophore called pyoverdine I (PVDI) [5,6] and a chelator of the hydroxyphenyl-thiazolinyl-thiazolidin type called pyochelin (PCH) [7]. The biochemistry and enzymology of PVDI and PCH biosynthesis has been best described in *P. aeruginosa* strain PAO1 and has been the object of several reviews in recent years [8,9]. In the last 2 years, important new insights have been obtained concerning bacterial subcellular organization and the distribution into particular areas of the cytoplasm, or within the membrane, of many enzymes involved in these siderophore biosynthesis processes. These findings reveal a higher degree of intracellular organization for siderophore biosynthesis in bacteria than previously suspected and give a completely new cellular view of these biosynthesis pathways. This work on the cellular organization of siderophore biosynthesis has mostly involved PVDI in *P. aeruginosa* PAO1, but data for PCH (in *P. aeruginosa*) and enterobactin (in *Escherichia coli*) biosynthesis are also available. The diverse data indicate that the enzymes involved in siderophore assembly can interact with the bacterial inner membrane and may form siderophore-specific multi-enzymatic complexes called siderosomes, the sites of assembly of the different siderophores in bacteria. Epifluorescence microscopy imaging showed that these enzymes are concentrated at the bacterial poles, suggesting that siderophore biosynthesis (siderosomes) are mostly located and active in these bacterial areas. This review summarizes these new findings and presents a cellular overview of siderophore biosynthesis in *P. aeruginosa*.

2. PVDI and PCH structure and chelating properties

PVDI is the archetype of a large family of siderophores called pyoverdines, made up of more than 60 different compounds, all produced by different strains and species of Pseudomonas [10–14]. All these siderophores are composed of three parts: (i) a chromophore

* Corresponding author at: UMR 7242, IREBS, ESBS, Blvd Sébastien Brant, BP 10412, Illkirch, Strasbourg F-67413, France. Tel.: +33 3 68 85 47 19; fax: +33 3 68 85 48 29.
E-mail address: isabelle.schalk@unistra.fr (I.J. Schalk).

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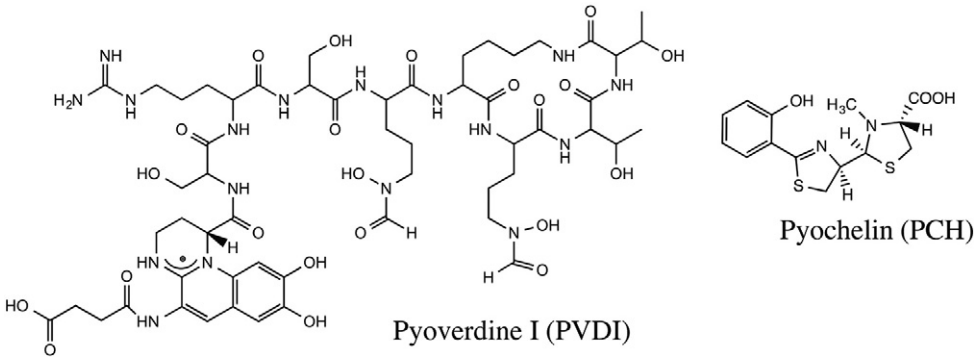

Fig. 1. The structures of pyoverdine I (PVDI) and pyochelin (PCH).

derived from 2,3-diamino-6,7-dihydroxyquinoline, which confers color and fluorescence to the molecule; (ii) a peptide moiety attached to the non-aromatic ring of the chromophore; and (iii) a dicarboxylic acid, amide or α-ketoglutaric acid attached to the C-3 of the chromophore. The amino acid composition and the length (usually between 6 and 12 residues) of the peptide moieties are very diverse and characteristic of each pseudomonad species [10–14]. This peptide chain may contain unusual amino acids, such as D-isomers, and those not usually found in proteins. In some pyoverdines like in PVDI produced by *P. aeruginosa* PAO1, the peptide moiety is cyclic. The PVDI structure (Fig. 1) has been solved by mass spectrometry and NMR [13,15]: it is composed of a partially cyclized octapeptide attached to the 2,3-diamino-6,7-dihydroxyquinoline-based chromophore. It chelates iron with a stochiometry of 1:1 (PVDI:Fe(III)), *via* the cathechol group of the chromophore and the two hydroxyornithines of the peptide moiety, and with a Ka of $10^{30.8}$ M^{-1} [16].

The structure of PCH (Fig. 1), the second siderophore produced by *P. aeruginosa*, is (4′*R*, 2″*R*, 4″*R*)-2′-(2-hydroxyphenyl)-3″-methyl-4′,5′,2″,3″,4″,5″-hexahydro-[4′,2″]bithiazolyl-4″-carboxylic acid with three chiral centers at positions C4′, C2″ and C4″ [7]. PCH chelates Fe^{3+} with an affinity of $10^{28.8}$ M^{-2} [17] and a 2:1 (PCH:Fe(III)) stoichiometry [18]: a tetra-dentate is provided by one molecule of PCH and a bi-dentate by the second PCH to complete the hexacoordinate octahedral geometry necessary for Fe^{3+} chelation [18].

3. The biochemistry of the PVDI biosynthesis pathway in *P. aeruginosa*

PVDI biosynthesis is a multistep process, starting in the bacterial cytoplasm and ending in the periplasm, and involving at least eleven different proteins. The steps of PVDI biosynthesis are briefly presented here (Fig. 2), but more details can be found in the review by Schalk and Guillon [8]. The backbone of PVDI is a peptide of 11 amino acids, with the sequence L-Glu–L-Tyr–D-Dab–L-Ser–L-Arg–L-Ser–L-fOHOrn–L-Lys–L-fOHOrn–L-Thr–L-Thr and which is assembled as a precursor in the bacterial cytoplasm. The first amino acid of the peptide (L-Glu) is the precursor of the α-ketoglutaric acid, succinamide or malamide side chains bound on C3 of the PVDI chromophore. The adjacent L-Tyr and D-Dab (D-amino butyric acid) residues form the chromophore and the other eight residues the peptide moiety. This PVDI precursor is assembled in the cytoplasm from the N- to the C-terminal end by the coordinated action of four nonribosomal peptide synthetases (NRPSs): PvdL, PvdI, PvdJ and PvdD [19–21]. Also involved indirectly in this process are three other enzymes (PvdA, PvdF and PvdH) producing the two atypical amino acids (D-Dab and L-fOHOrn (L-formyl-N^{δ}-hydroxyornithine)) present in the PVDI peptide precursor [22]. The L-ornithine hydroxylase PvdA and the hydroxyornithine transformylase PvdF catalyze the transformation of L-ornithine into L-formyl-N^{δ}-hydroxyornithine, which is then incorporated into the peptide chain by the NRPSs PvdI and PvdJ [23,24]. The amidotransferase PvdH is involved in the synthesis of D-Dab, one of the substrates of PvdL [25].

PvdL, PvdI, PvdJ and PvdD are typical NRPSs with a multimodular architecture. Each module catalyzes the incorporation of one specific amino acid substrate into the peptide product and the formation of peptide bonds between the amino acids. PvdL, the NRPS involved in the assembling of the tripeptide L-Glu–L-Tyr–D-Dab, is atypical of PVDI NRPSs because the initial module—PvdL-M1—contains an unusual domain, very similar to acyl coenzyme A ligases [20], and able to bind to myristate [26]. Consistent with this observation, it has recently been proposed that PVDI synthesis starts with PvdL, which couples coenzyme A to a myristic or myristoleic acid in an ATP-dependent reaction, and delivers the complex formed to the L-Glu residue carried by the second module of PvdL [20,26,27]. In the two subsequent steps, D-Tyr and L-Dab are incorporated by the two other modules of PvdL, forming the precursor of the dihydroxyquinoline chromophore [20]. Next, L-Ser, L-Arg, L-Ser and L-OHOrn are incorporated successively by PvdI, L-Lys and L-OHOrn by PvdJ and the two L-Thr by PvdD [22]. The presence of a myristate or myristoleate chain on the first residue (L-Glu) keeps the PVDI precursor bound at the inner membrane throughout the cytoplasmic biosynthetic process [27], thereby preventing its diffusion across the bacterial cytoplasm; such diffusion may be problematic because this molecule is able to chelate iron and other metals [27,28]. This 11-amino acid peptide with either a myristate or myristoleate chain and a unformed chromophore is believed to be the cytoplasmic precursor of PVDI, and its structure has been solved by mass spectrometry [27].

This PVDI precursor contains the complete backbone of PVDI; it is transported across the inner membrane by an ABC export transporter, PvdE, and undergoes different maturation steps in the periplasm [28]. PvdQ, an N-terminal nucleophile periplasmic hydrolase, is involved in the excision of the fatty-acid chain (myristate or myristoleate chain) resulting in the PVDI precursor called ferribactin [26,27]. The chromophore is then cyclized by the tyrosinase PvdP, which converts the non-fluorescent ferribactin, into the fluorescent PVDI [29]. The periplasmic enzymes PvdN and PvdO [21,30] are also necessary for PVDI production [28,31–33]: mutation of PvdN and PvdO abolishes the secretion of fluorescent PVDI [28]. However, the exact functions of these enzymes are still unknown. Possibly, they convert the L-Glu side chain, after removing the myristic or myristoleic acid, into the various side chains found in PVDI [11].

4. The biochemistry of PCH biosynthesis pathway in *P. aeruginosa*

PCH is the condensation product of salicylate and two molecules of cysteine (Fig. 3) [9]. The first step is the conversion of chorismate into salicylate *via* isochorismate by the isochorismate synthetase PchA [34,35] and the isochorismate pyruvate-lyase PchB [35,36]. Then, the salicylate molecule is activated by PchD and loaded onto the NRPS PchE, and the enzyme links this compound to a first L-Cys to form Dha