siderophore intracellularly, and the fate of intracellular siderophore are issues that have not been resolved and await further study.

References


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A new mechanism for membrane iron transport in Pseudomonas aeruginosa

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Abstract

Various biochemical and biophysical studies have demonstrated the existence of a novel iron-uptake mechanism in Pseudomonas aeruginosa, different from that generally described for ferrichrome and ferric-enterobactin in Escherichia coli. This new iron-uptake mechanism involves all the proteins generally reported to be involved in the uptake of ferric-siderophore complexes in Gram-negative bacteria (i.e. the outer membrane receptor, periplasmic binding protein and ATP-binding-cassette transporter), but differs in the behaviour of the siderophore. One of the key features of this process is the binding of iron-free pyoverdin to the outer membrane receptor FpvA in conditions of iron deficiency.

Introduction

Pseudomonas aeruginosa is considered to be an important opportunistic pathogen, highly patho-
genic for individuals with compromised immunity. In this bacterium, the expression of relevant virulence factors is tightly controlled by the iron level. In response to iron limitation, the bacterium synthesizes a major siderophore called pyoverdin (PaA) [1]. In this review we describe recently published results concerning the mechanism of iron uptake via PaA in P. aeruginosa. These new findings concern the interactions between PaA and its outer membrane receptor, FpvA [2].

**PaA**

PaA is synthesized in iron-deficient conditions and the binding constant for its association with Fe$^{3+}$ is extremely high (around 10^{22} M^{-1}). This siderophore possesses a chromophore derived from 2,3-diamino-6,7-dihydroxyquinoline, which confers colour and fluorescence to the molecule, linked to a partially cyclic octapeptide (Figure 1) [3]. Fe$^{3+}$ is complexed by PaA via three bidentate groups, one belonging to the chromophore and two to the peptide.

**PaA and PaA-Fe binding to the outer membrane receptor FpvA**

The fluorescence resonance energy transfer (FRET) between iron-free PaA and the Trp of FpvA has clearly shown that, under iron limitation, PaA-loaded FpvA is the normal state of the receptor in vivo [4,5]. Moreover, under such iron conditions, all receptor-binding sites at the cell surface are occupied by iron-free PaA [4,5]. It has also been suggested that such a loading status for a siderophore outer membrane receptor exists both in Escherichia coli and Aeromonas hydrophila [6].

In experiments with radiolabelled siderophores, we observed a 30-fold difference in the affinities of PaA ($K_{d_{app}} = 30$ nM; where $K_{d_{app}}$ is the apparent dissociation constant) and PaA-Fe ($K_{d_{app}} = 1.0$ nM) for FpvA in vivo and a 17-fold difference (PaA, $K_{d_{app}} = 17$ nM; PaA-Fe, $K_{d_{app}} = 1.4$ nM) in vivo in the absence of TonB [5]. TonB has no effect on the affinity of PaA-Fe. It simply doubles the affinity of PaA. Similar affinities of iron-free siderophore and ferric-siderophore for their receptors have been reported in A. hydrophila [6]. Without doubt, such a small difference between the affinities of PaA and PaA-Fe for FpvA was very surprising. Under iron limitation, the free form of the siderophore is always in large excess in the medium with respect to the iron-loaded siderophore. It is difficult to understand how an efficient iron-uptake mechanism can cope with a receptor displaying only small differences in affinity for iron-loaded and iron-free siderophore.

Moreover, time-resolved fluorescence has shown that the FpvA–PaA complex is able to adopt two different conformations [7]. In both conformers, the dihydroquinoline moiety of the PaA is fully protonated or co-ordinated by protein-charged groups, but the polarity of its environment, its solvent accessibility and its rotational dynamics differ between conformers. In the presence of metal (FpvA–PaA-Ga and FpvA–PaA-Al), the solvent accessibility and mobility of the dihydroquinoline moiety are intermediate between those observed for the two FpvA–PaA conformers [7]. Thus, the binding interactions between ferric-PaA and apo-PaA and their outer membrane receptor FpvA seem to be quite complex. These interactions may involve more than one binding site per receptor or different receptor–ligand conformations. Mutagenesis studies on FepA have also suggested that there may be dual...
binding sites for ferric-enterobactin, with the secondary site located deeper within the protein [8,9].

**Ferric-PaA uptake through the outer membrane**

During iron uptake, the bound iron-free PaA of the FpvA-PaA complex is displaced by the extracellular tritiated ferric-PaA in *P. aeruginosa* [4,5]. PaA-Fe does not act as an iron donor by the mechanism described for *A. hydrophila* [6]. The kinetics of formation of this FpvA-PaA-Fe complex during iron uptake are regulated by TonB [5], suggesting that TonB plays a critical role in the formation of the receptor–siderophore–iron complex.

The formation of the receptor–siderophore–iron complex is the first step in iron uptake. Three different mechanisms have been described for ferric-siderophore uptake in Gram-negative bacteria (Figure 2). The first is that described for ferrichrome in *E. coli*. In this mechanism, the ferrichrome-siderophore complex binds to its outer membrane receptor and is transported into the cytoplasm without any siderophore-iron dissociation (Figure 2). In the two other mechanisms, the outer membrane receptor is already loaded with a molecule of apo-siderophore. In mechanism 3, found in *E. coli* and *A. hydrophila* [6], the extracellular ferric-siderophore acts as an iron donor and brings the iron ion to the apo-siderophore already bound to the outer membrane receptor (Figure 2). In the mechanism studied by our group (mechanism 2 in Figure 2), the extracellular ferric-siderophore displaces the already bound iron-free siderophore on the receptor. In this case, iron is not transferred between siderophores; instead, an iron-loaded siderophore displaces an iron-free siderophore. This major difference between mechanisms 2 and 3 (Figure 2) is consistent with the specificity of the outer membrane receptors involved in each mechanism. In our case (mechanism 2), the outer membrane PaA receptor is highly strain-specific [10]. Conversely, in mechanism 3, the outer membrane receptor has very low siderophore specificity and recognizes a wide variety of siderophores [6].

**PaA recycling after ferric-PaA uptake**

Our group recently showed that after the uptake of iron and its release into the cells, the iron-free PaA

![Figure 2](image-url)

**Figure 2**

Mechanisms of formation of outer membrane receptor–siderophore–iron complexes during iron uptake

OM, outer membrane; OMR, outer membrane receptor; Sid, siderophore.
Biological function of PaA binding to the FpvA receptor

The biological function of PaA binding to FpvA in the absence of iron is unknown. However, several observations suggest that this binding may be involved in transcriptional activation similar to that described for FecA [13]. Indeed, it has been shown that the presence of PaA in the extracellular medium positively regulates expression of the \( \text{fpxA} \) and \( \text{fpxA}_{BCD} \) genes [14] via a sigma factor called PvdS [15]. We have also shown that FpvA, like FecA, has an extended N-terminus [16].

Conclusions

The iron-uptake mechanism described here for PaA-Fe is clearly different from those proposed for ferrichrome in \( \text{E. coli} \) or for siderophores of \( \text{A. hydrophila} \). The major difference is the loading status of the outer membrane receptor in the absence of iron (FpvA-PaA). To date, investigations have concentrated on the interactions between PaA or PaA-Fe and the outer membrane receptor FpvA. Other differences may also exist in the interactions of PaA-Fe with the periplasmic binding protein and the ATP-binding-cassette transporter involved in the transport of PaA-Fe through the inner membrane.

References


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Transferrin-mediated iron acquisition by pathogenic Neisseria

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Abstract

The pathogenic \textit{Neisseria} have a siderophore-independent iron-uptake system reliant on a direct interaction between the bacterial cell and transferrin. In the meningococcus this uptake system is dependent on two surface-exposed transferrin-binding proteins. This short account will review our current knowledge of the transferrin-mediated iron-acquisition system of pathogenic \textit{Neisseria}.

Introduction

\textit{Neisseria meningitidis} is the causative agent of bacterial meningitis. Although a vaccine is now available for the C strain no vaccine is available for the B strain, the most prevalent in the Western world. Under iron-limiting conditions \textit{N. meningitidis} expresses a number of surface receptors for host iron-containing proteins, including...