

GUEST COMMENTARIES

Unexpected Interaction of a Siderophore with Aluminum and Its Receptor[∇]

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Pseudomonas aeruginosa is an important opportunistic pathogen, and one of the major factors contributing to its virulence is the capacity to produce a high-affinity iron-scavenging siderophore, pyoverdine (10, 11, 21). Pyoverdines form a family of peptidic siderophores comprising a variable peptide chain and a chromophore conferring the characteristic fluorescence of the apo-pyoverdine (11, 15, 21). Pyoverdines not only are siderophores but also can be considered to be authentic signal molecules, since the interaction of ferripyoverdine with the FpvA receptor triggers a signaling cascade for the production of virulence factors such as exotoxin A and the protease PrpL (9, 20, 21). Furthermore, the pyoverdine-mediated iron uptake system is important for the formation of biofilms by *P. aeruginosa* (1, 8). The FpvA receptor has been copurified with pyoverdine and its three-dimensional structure determined, confirming its association with what was then thought to be apo-pyoverdine because of its strong fluorescence (3, 7). In this issue, Greenwald et al. show that this molecule associated with FpvA is not apo-pyoverdine but Al-pyoverdine (6). As we will see, this discovery changes the way ferripyoverdine uptake is considered to occur (Fig. 1).

SIDEROPHORES DO NOT BIND ONLY IRON

Siderophores are able to bind metals other than iron, as has been shown for the second siderophore of *P. aeruginosa*, pyochelin (19), and this is also the case for pyoverdines. Indeed, pyoverdine was found to bind gallium and vanadium, and these complexes can inhibit the growth of *P. aeruginosa* (2, 6, 8). Ga in particular interferes with Fe-dependent processes, because unlike Fe³⁺, Ga³⁺ cannot be reduced (6, 8, 15). Exposure to subinhibitory concentrations of Ga caused a drop in the expression of *pvdS*, encoding the extracytoplasmic function sigma factor, which controls the transcription of pyoverdine biosynthesis genes (8). Pyoverdines can also complex aluminum with high affinity (4) and were also found to complex copper (22), and it has been recently demonstrated that excess copper induces a higher-level expression

of pyoverdine genes (18). These results and others suggest that high-affinity siderophores such as pyoverdine could be involved in the sequestration of toxic metals.

MECHANISM OF FERRIPYOVERDINE UPTAKE

Uptake of ferripyoverdine begins at the level of the TonB-dependent receptor FpvA, a gated β -barrel porin whose structure has recently been determined (3). The ferripyoverdine receptor, FpvA, has until now been thought to be associated with the apo form of pyoverdine, and the crystal structure of FpvA indeed seemed to confirm such an association (3, 13, 15). Following this observation, a mechanism of pyoverdine-mediated iron transport in *P. aeruginosa* was proposed (13). Apo-pyoverdine was described to be bound to the surface of the FpvA receptor in a pocket lined with aromatic residues (3, 13, 17). It has therefore been proposed that iron-loaded pyoverdine can exchange with unloaded pyoverdine at the surface of the receptor, a process which is dependent on the inner membrane protein TonB, which relays the proton motive force to FpvA (16). Here, Greenwald et al. reveal that in reality apo-pyoverdine has no affinity at all for the FpvA receptor and that the previously observed fluorescence rather is due to the association of Al-pyoverdine with the receptor, meaning that the interactions measured by fluorescent resonance energy transfer are in fact those occurring between Al-pyoverdine and FpvA (12, 13). When the growth medium was treated with a metal binding IMAC resin to remove all traces of metals, no association of apo-pyoverdine with the receptor could be detected. In contrast to iron, which quenches the fluorescence of pyoverdine, Al enhances it (6), explaining why the form of pyoverdine bound to FpvA was originally thought to be apo-pyoverdine.

FATE OF FERRIPYOVERDINE AND RECYCLING OF APO-PYOVERDINE

Another interesting observation made here is the fact that pyoverdine can mediate the transport of not only Fe but also Al and Ga to the periplasm, while apo-pyoverdine was not found in the periplasmic compartment. However, the proportion of Ga-pyoverdine in the periplasm is higher than

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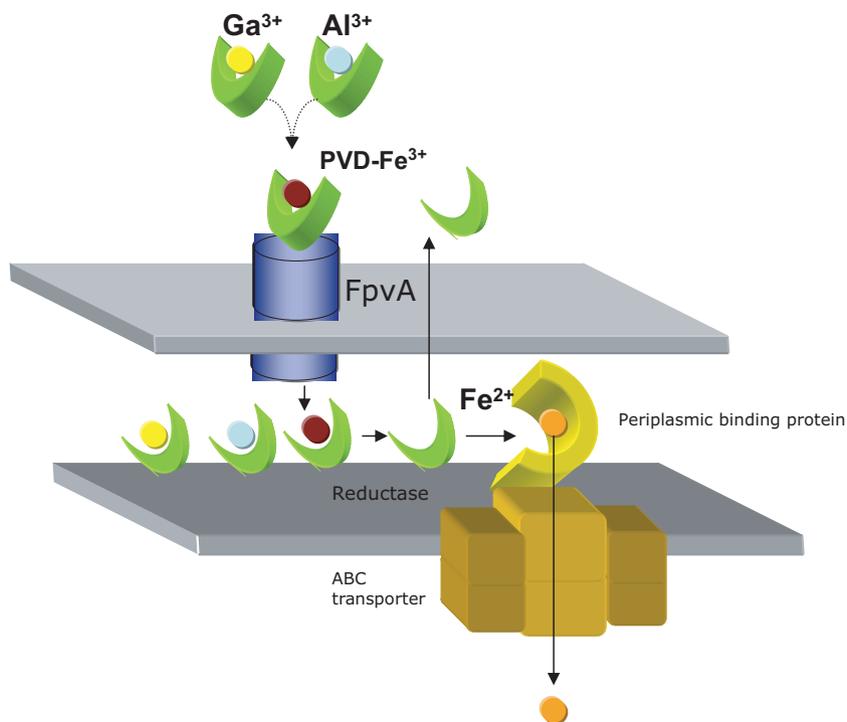


FIG. 1. Hypothetical model of ferripyoverdine uptake by FpvA (TonB and ExbBD are not shown for clarity) based on the discovery of rapid recycling of pyoverdine (PVD), suggesting that it is not transported to the cytoplasm (5, 14, 15). Ferripyoverdine is bound to FpvA and is transported via the proton motive force relayed by TonB; once it is in the periplasm, a still-undescribed reduction process takes place and Fe^{2+} is bound to a periplasmic binding protein which delivers it to an ABC transporter. Ga-pyoverdine and Al-pyoverdine also can be transported but are not further processed in the periplasm.

that of Al-pyoverdine, suggesting that the transport of Ga mediated by pyoverdine is more efficient. We also do not yet know what happens to ferripyoverdine after binding to the receptor (Fig. 1). There is no clear evidence for a periplasmic protein binding pyoverdine so far and also no evidence for an inner membrane transporter (8, 11). Finally, one interesting question is how iron is removed from ferripyoverdine and how this process takes place in the periplasm. Since pyoverdine is rapidly recycled, this indeed strongly suggests a periplasmic localization of the reduction process (5, 6, 14).

REFERENCES

- Banin, E., M. L. Vasil, and E. P. Greenberg. 2005. Iron and *Pseudomonas aeruginosa* biofilm formation. *Proc. Natl. Acad. Sci. USA* **102**:11076–11081.
- Baysse, C., D. De Vos, Y. Naudet, A. Vandermonde, U. Ochsner, J. M. Meyer, H. Budzikiewicz, M. Schafer, R. Fuchs, and P. Cornelis. 2000. Vanadium interferes with siderophore-mediated iron uptake in *Pseudomonas aeruginosa*. *Microbiology* **146**:2425–2434.
- Cobessi, D., H. Celia, N. Folschweiller, I. J. Schalk, M. A. Abdallah, and F. Pattus. 2005. The crystal structure of the pyoverdine outer membrane receptor FpvA from *Pseudomonas aeruginosa* at 3.6 angstroms resolution. *J. Mol. Biol.* **347**:121–134.
- del Olmo, A., C. Caramelo, and C. SanJose. 2003. Fluorescent complex of pyoverdine with aluminum. *J. Inorg. Biochem.* **97**:384–387.
- Greenwald, J., F. Hoegy, M. Nader, L. Journet, G. L. Mislin, P. L. Graumann, and I. J. Schalk. 2007. Real time fluorescent resonance energy transfer visualization of ferric pyoverdine uptake in *Pseudomonas aeruginosa*: a role for ferrous iron. *J. Biol. Chem.* **282**:2987–2995.
- Greenwald, J., G. Zeder-Lutz, A. Hagege, H. Celia, and F. Pattus. 2008. The metal dependence of pyoverdine interactions with its outer membrane receptor FpvA. *J. Bacteriol.* **190**:6548–6558.
- Hoegy, F., H. Celia, G. L. Mislin, M. Vincent, J. Gallay, and I. J. Schalk. 2005. Binding of iron-free siderophore, a common feature of siderophore outer membrane transporters of *Escherichia coli* and *Pseudomonas aeruginosa*. *J. Biol. Chem.* **280**:20222–20230.
- Kaneko, Y., M. Thoendel, O. Olakanmi, B. E. Britigan, and P. K. Singh. 2007. The transition metal gallium disrupts *Pseudomonas aeruginosa* iron metabolism and has antimicrobial and antibiofilm activity. *J. Clin. Investig.* **117**:877–888.
- Lamont, I. L., P. A. Beare, U. Ochsner, A. I. Vasil, and M. L. Vasil. 2002. Siderophore-mediated signaling regulates virulence factor production in *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci. USA* **99**:7072–7077.
- Meyer, J. M., A. Neely, A. Stintzi, C. Georges, and I. A. Holder. 1996. Pyoverdine is essential for virulence of *Pseudomonas aeruginosa*. *Infect. Immun.* **64**:518–523.
- Ravel, J., and P. Cornelis. 2003. Genomics of pyoverdine-mediated iron uptake in pseudomonads. *Trends Microbiol.* **11**:195–200.
- Schalk, I. J., P. Kyslik, D. Prome, A. van Dorselaer, K. Poole, M. A. Abdallah, and F. Pattus. 1999. Copurification of the FpvA ferric pyoverdine receptor of *Pseudomonas aeruginosa* with its iron-free ligand: implications for siderophore-mediated iron transport. *Biochemistry* **38**:9357–9365.
- Schalk, I. J., C. Hennard, C. Dugave, K. Poole, M. A. Abdallah, and F. Pattus. 2001. Iron-free pyoverdine binds to its outer membrane receptor FpvA in *Pseudomonas aeruginosa*: a new mechanism for membrane iron transport. *Mol. Microbiol.* **39**:351–360.
- Schalk, I. J., M. A. Abdallah, and F. Pattus. 2002. Recycling of pyoverdine on the FpvA receptor after ferric pyoverdine uptake and dissociation in *Pseudomonas aeruginosa*. *Biochemistry* **41**:1663–1671.
- Schalk, I. J. 2008. Metal trafficking via siderophores in Gram-negative bacteria: specificities and characteristics of the pyoverdine pathway. *J. Inorg. Biochem.* **102**:1159–1169.
- Schons, V., R. A. Atkinson, C. Dugave, R. Graff, G. L. Mislin, L. Rochet, C. Hennard, B. Kieffer, M. A. Abdallah, and I. J. Schalk. 2005. The structure activity relationship of ferric pyoverdine bound to its outer membrane transporter: implications for the mechanism of iron uptake. *Biochemistry* **44**:14069–14079.
- Shen, J. S., V. Geoffroy, S. Neshat, Z. Jia, A. Meldrum, J. M. Meyer, and K. Poole. 2005. FpvA-mediated ferric pyoverdine uptake in *Pseudomonas aeruginosa*: identification of aromatic residues in FpvA implicated in ferric pyoverdine binding and transport. *J. Bacteriol.* **187**:8511–8515.
- Teitzel, G. M., A. Geddie, S. K. De Long, M. J. Kirisits, M. Whiteley, and M. R. Parsek. 2006. Survival and growth in the presence of elevated copper:

- transcriptional profiling of copper-stressed *Pseudomonas aeruginosa*. J. Bacteriol. **188**:7242–7256.
19. **Visca, P., G. Colotti, L. Serino, D. Verzili, N. Orsi, and E. Chiancone.** 1992. Metal regulation of siderophore synthesis in *Pseudomonas aeruginosa* and functional effects of siderophore-metal complexes. Appl. Environ. Microbiol. **58**:2886–2893.
20. **Visca, P., L. Leoni, M. J. Wilson, and I. L. Lamont.** 2002. Iron transport and regulation, cell signalling and genomics: lessons from *Escherichia coli* and *Pseudomonas*. Mol. Microbiol. **45**:1177–1190.
21. **Visca, P., F. Imperi, and I. L. Lamont.** 2007. Pyoverdine siderophores: from biogenesis to biosignificance. Trends Microbiol. **15**:22–30.
22. **Xiao, R., and W. S. Kisaalita.** 1995. Purification of pyoverdines of *Pseudomonas fluorescens* 2-79 by copper-chelate chromatography. Appl. Environ. Microbiol. **61**:3769–3774.

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