Exogenous siderophore-mediated iron uptake in *Pseudomonas aeruginosa*: possible involvement of porin OprF in iron translocation

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In addition to the two siderophores pyoverdine and pyochelin synthesized by *Pseudomonas aeruginosa* ATCC 15692 (strain PAO1), several siderophores produced by other bacteria or fungi, namely cepabactin, salicylic acid, desferriferichrysin, desferriferrirocine, desferriferrioxamine B, desferriferrioxamine E and coprogen, were able to promote iron uptake with variable efficiencies into this bacterium. For most of these siderophores, these results were consistent with the growth stimulation produced by the same compounds in a plate bioassay. Desferriferriochrome A, enterobactin and desferriferri-rubin, however, did not promote iron uptake, although enterobactin and desferriferri-rubin stimulated bacterial growth. These paradoxical data are discussed in view of siderophore-inducible iron uptake systems, as demonstrated recently for enterobactin. Among the strains tested, including the wild-type PAO1, the pyoverdine-less mutant PAO6606 and the two porin-mutants *P. aeruginosa* H636 (*oprF*:: Ω) and *P. aeruginosa* H673 (*oprD*::Tn501), only for the porin-OprF mutant were fewer siderophores able to promote iron uptake compared to the other strains. Such results suggest that beside specific routes for iron uptake *P. aeruginosa* is also able to take up siderophore-liganded iron through OprF.

Introduction

To fulfill their iron requirement, many micro-organisms have evolved specialized molecules named siderophores which, when excreted in the cell environment, efficiently solubilize iron due to their high binding capacities for this element (Neilands, 1981; Matzanke *et al.*, 1989). The ferrisiderophore complex is then usually translocated through the cell membranes by a highly specific active transport system involving several specialized proteins, the best characterized being the iron-regulated outer-membrane proteins (IROMPs) which act as ferrisiderophore receptors (Neilands, 1982). Iron-starved *Escherichia coli* cells synthesize several such IROMPs, each recognizing usually one (ferri)siderophore, including the endogenous siderophore produced by this bacterium, enterobactin, as well as the iron complexes of desferriferriochrome, desferriferri-chrysin, coprogen or rhodotorulic acid, siderophores synthesized by other micro-organisms (Braun *et al.*, 1987). Other bacteria, besides *E. coli*, can

acquire iron from exogenous ferrisiderophores. For example, *Mycobacterium smegmatis* incorporates iron liganded to rhodotorulic acid (Ratledge, 1987), while *Erwinia herbicola* efficiently incorporates iron complexed to enterobactin, coprogen and, to a lesser extent, desferriferriochrome in addition to its own siderophore, desferriferrioxamine E (Berner *et al.*, 1988). Also, growth of some *Haemophilus* strains is stimulated by enterobactin (Williams *et al.*, 1990), whereas this compound induces in *Pseudomonas aeruginosa* a new iron uptake system specific for ferrienterobactin (Poole *et al.*, 1990). *P. aeruginosa* was also seen to recognize, together with its own (ferri)siderophore pyoverdine_{PAO}, the iron complexes of pyoverdines synthesized by other strains of fluorescent pseudomonads, such as pyoverdine_{Pch} produced by *P. chlororaphis* ATCC 9446 and pyoverdine_{PN13525} produced by *P. fluorescens* ATCC 13525, thus demonstrating an exception to the general rule of strict specificity of recognition established for many different *Pseudomonas* species with regard to their respective pyoverdine-mediated iron uptake capabilities (Hohnadel & Meyer, 1988).

It is of some interest to define the iron metabolism and especially the iron incorporation potentials that an opportunistic pathogen like *P. aeruginosa* may develop

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Abbreviation: EDDHA, ethylenediamine di(hydroxyphenylacetic acid).

(Bozenhart & Ruden, 1987). In this paper the potential of 13 exogenous siderophores and citrate to promote iron uptake in *P. aeruginosa* is described. In addition, the question of whether these siderophore-iron complexes utilize one of the known porin pathways, OprF (Nikaido & Hancock, 1986; Woodruff & Hancock, 1988) or OprD (Trias & Nikaido, 1990), as opposed to specific siderophore receptors, is explored.

Methods

Bacterial strains. The bacterial strains used in this study were the wild-type strain *P. aeruginosa* ATCC 15692 (strain PAO1) and three mutants derived originally from this strain. The pyoverdine-less mutant PAO6606, obtained by UV-mutagenesis, has been characterized previously (Hohnadel *et al.*, 1986). The two porin-mutants, strains H636 and H673, were kindly provided by R. E. W. Hancock (University of British Columbia, Canada). Strain H673 is a *oprD::Tn501* mutant which lacks the outer-membrane protein D2 (H. Meadows & R. E. W. Hancock, unpublished data), whereas strain H636 is a *oprF::Ω* mutant lacking the outer-membrane protein F (Woodruff & Hancock, 1988).

Plate bioassays. The influence of siderophores on bacterial growth was studied by a disk diffusion method on solid media. Plate bioassays were performed on nutrient agar and on succinate-agar media supplemented or not with 100 µg ml⁻¹ of purified ethylenediamine di(hydroxyphenylacetic acid) [EDDHA], as previously described (Hohnadel & Meyer, 1988). Succinate-agar media were supplemented with 1 mM-methionine for growth of the methionine auxotroph strain PAO6606. Cells harvested from an overnight culture in the corresponding liquid medium were washed twice with sterile saline and plated evenly with the help of sterile glass beads at a concentration of 10⁶ cells per plate. Sterile paper disks were impregnated with 20 µl of filter-sterilized 1 mM-siderophore solutions (3 mM for siderophores forming a 3:1 complex with iron, i.e. cepabactin) and deposited at the surface of the inoculated agar, four to five different compounds being tested per plate. Triplicate independent assays were run for each strain and for the different siderophores tested. Bacterial growth was observed at various times during incubation of the plates at 37 °C, and stimulation effects were visualized by development of the bacterial lawn first around the impregnated paper disks. This occurred, approximately 6 h after plating on nutrient agar without EDDHA, but required an 18 to 24 h incubation for growth to be visible on succinate-agar medium supplemented with EDDHA. No attempts were made to quantify the growth stimulation effects of the siderophores tested since, due to differences in the solubility of these compounds in aqueous media (e.g. pyoverdines are highly soluble in water, whereas for preparation of 1 or 3 mM solutions of cepabactin, enterobactin or pyochelin, methanol was required), the diameter of the stimulation zone measured around the disks may not correlate with the potency of the siderophores. To prevent any growth inhibitory effect due to methanol for compounds dissolved in this solvent, the impregnated disks were first air-dried before being deposited on the surface of the plates.

⁵⁹Fe incorporation. The protocol used has been described previously (Cornelis *et al.*, 1989). Briefly, cells harvested from overnight cultures in succinate medium at 37 °C (1 mM-methionine/succinate medium for strain PAO6606) were washed twice in sterile saline and suspended in uptake medium (succinate medium with the nitrogen source omitted) at OD₆₀₀ = 3.0. Uptake assays were run in 250 ml Erlenmeyer flasks maintained at 30 °C in a shaking (100 r.p.m.) waterbath and containing 8 ml of uptake medium and 1 ml of bacterial suspension. After 10 min,

uptake was initiated by adding 1 ml of a mixture consisting of 5 µl of ⁵⁹Fe [commercial solution: chloride form, specific activity 20 mCi mg⁻¹ (740 MBq mg⁻¹); CEA, France], 50 µl of a 1 mM (or 3 mM; see above) siderophore solution and 945 µl of uptake medium added 15 min after mixing the two first solutions. Samples (1 ml) of bacterial suspension were withdrawn at various times during the 15 min incubation period and filtered through Millipore filters (0.22 µm). Filters were then washed twice with 2 ml of uptake medium before being counted for radioactivity using a Beckmann Gammamatic 4000 counter. Assays without cells were performed as controls for the full solubilization of iron by the siderophores during the 15 min incubation time. All assays were run in triplicate for each ligand tested and data are usually presented as means of the three experimental values.

Siderophores and chemicals. The siderophores tested in this study included ferrirubin, coprogen and ferrichrysin, kindly provided by Dr N. A. C. Curtis (ICI, UK) and ferricrocin and ferrichrome A, a gift from Dr G. Winkelmann (Tübingen University, Germany). All these compounds, supplied as iron complexes, were deferrated by a previously described method (Wiebe & Winkelmann, 1975) before their use in plate bioassays or in uptake studies. The different pyoverdines tested, originally from *P. aeruginosa* PAO1, *P. aeruginosa* ATCC 27853, *P. fluorescens* ATCC 13525, *P. fluorescens* ATCC 17400, *P. fluorescens* CCM 2798 and *P. chlororaphis* ATCC 9446, were purified according to previously published methods (Hohnadel & Meyer, 1988), as were other siderophores of *Pseudomonas* origin, including cepabactin from *P. cepacia* ATCC 25416 (Meyer *et al.*, 1989), pyochelin isolated either from *P. aeruginosa* PAO1 or from *P. cepacia* ATCC 25416 (Meyer *et al.*, 1989), and desferriferrioxamine E from *P. stutzeri* ATCC 27588 (Meyer & Abdallah, 1980). Enterobactin from *E. coli* strain AN311, kindly provided by Dr I. G. Young (Canberra University, Australia), was purified according to Young & Gibson (1979). Desferriferrioxamine B (Desferal; Ciba-Geigy, Switzerland), salicylic acid and citric acid were of commercial origin.

Results

Cross-feeding assays

As visualized by plate bioassays, many siderophores of various bacterial origins were able to promote growth of the wild-type *P. aeruginosa* strain PAO1 (Table 1). Some of them, i.e. the endogenous pyoverdine of *P. aeruginosa* PAO1 as well as the pyoverdines of *P. fluorescens* ATCC 13525 and *P. chlororaphis* ATCC 9446, resulted in a strong stimulatory effect as judged by the bacterial lawn which developed first around the respective siderophore-impregnated paper disks. Incubation at 37 °C for 2 d was necessary for bacterial growth on the entire surface of the EDDHA/succinate agar plates to be visible, whereas some growth was already visible around the disks after a 12 h incubation period and had reached its maximal intensity 12 h later. In contrast, bacterial growth in the immediate vicinity of disks impregnated with the pyoverdines of *P. fluorescens* ATCC 17400, *P. fluorescens* CCM 2798 or *P. aeruginosa* ATCC 27853 required, like growth on the entire surface of the plates, 48 h to develop. The other siderophores tested, chemically unrelated to pyoverdines, resulted in various responses:

Table 1. Growth stimulation of the different *P. aeruginosa* strains mediated by various siderophores

The siderophores were used in this study as the deferrated forms. Growth effects were observed after 24 h at 37 °C on EDDHA-supplemented succinate-agar medium and are indicated as follows: +, growth stimulation; 0, no stimulation; +/–, variable responses (slight stimulation or no stimulation) observed among the triplicate independent experiments performed.

Siderophore	Growth stimulation of strain:			
	PAO1	PAO6606	H636	H673
Ferricrocin	+	+/-	0	0
Ferrichrysin	+	+	+	+
Ferrirubin	+	+	+	+
Ferrioxamine E	+	+	+	+
Ferrioxamine B	+	+	+	+
Ferrichrome A	0	0	0	0
Enterobactin	+	+	+	+
Pyochelin	+/-	0	+/-	0
Cepabactin	0	0	+/-	0
Pyoverdine _{PAO}	+	+	+	+
Pyoverdine _{Pa27853}	0	0	0	0
Pyoverdine _{Pn13525}	+	+	+	+
Pyoverdine _{Pch}	+	+	+	+
Pyoverdine _{Pn17400}	0	0	0	0
Pyoverdine _{Pn2798}	0	0	0	0

the deferrated forms of ferricrocin, ferrichrysin, ferrirubin, ferrioxamine E and ferrioxamine B slightly stimulated the growth of *P. aeruginosa* PAO1, since weak growth was visible around the respective disks after a 24 h incubation, whereas enterobactin resulted in a greater stimulatory effect, bacterial growth around the disk appearing earlier (about 18 h) and being more developed after 24 h incubation compared to growth with the other compounds. Desferriferriochrome A, as well as cepabactin, did not promote growth, whereas pyochelin led to variable results with one negative and two very slightly positive growth responses in the three experiments performed.

As seen in Table 1, only a few differences were observed with the other *P. aeruginosa* strains tested, when compared to the wild-type *P. aeruginosa* PAO1. The pyoverdine-deficient mutant PAO6606 responded to the different siderophores similarly to strain PAO1 with only two exceptions: desferriferrirocroc resulted in very poor stimulation of growth to no growth, whereas pyochelin did not stimulate growth in any of the three assays performed. The two porin-mutants, strains H636 and H673, also demonstrated only small changes in their respective responses to the siderophores compared to the two other strains, the main changes being that desferriferrirocroc did not promote the growth of these two strains, whereas in one of three experiments, cepabactin was seen to promote the growth of strain H636 slightly.

⁵⁹Fe uptake

The 13 iron complexes tested in iron uptake experiments performed on the wild type strain *P. aeruginosa* PAO1 could be classified in four groups, depending on the iron uptake kinetics experimentally observed (Fig. 1). The first group contained siderophores which very efficiently promoted iron uptake, namely the endogenous *P. aeruginosa* PAO1 siderophore, pyoverdine_{PAO}, and cepabactin, the major siderophore of *P. cepacia* ATCC 25416. The second group included citrate, salicylic acid, desferriferriochrysin and pyochelin, only one of which, pyochelin, is synthesized by *P. aeruginosa* PAO1. These ligands promoted iron incorporation at, on average, 30% of the efficiency obtained with the first group of siderophores. The third group, represented by desferriferrioxamine B, desferriferrioxamine E, desferriferrirocroc and coprogen, demonstrated only a weak ability to promote iron uptake, at 10 to 15% of the efficiency of pyoverdine_{PAO}. Finally, desferriferriochrome A, enterobactin and desferriferrirobin, constituting the fourth group, were unable to promote iron uptake in *P. aeruginosa* PAO1.

The same experiments, done with the pyoverdine-less mutant PAO6606, resulted in roughly similar grouping of the different siderophores tested with certain changes. As shown in Fig. 2, iron uptake efficiencies were significantly increased for cepabactin, pyochelin, salicylic acid and citrate. In contrast, the efficiency of pyoverdine-iron uptake was about 30% less. Desferriferriochrysin, together with the siderophores of the third group (desferriferrioxamines B and E, desferriferrirocroc and coprogen), demonstrated a similar capacity to promote iron uptake in PAO6606 compared to PAO1, and desferriferriochrome A, enterobactin and desferriferrirobin were again ineffective.

Iron uptake with the porin OprD (= protein D2) mutant (strain H673, Fig. 3) followed a similar pattern to that observed with PAO1: enhanced uptake with desferriferrioxamine B, decreased uptake with desferriferriochrysin, and no uptake with desferriferrioxamine E were the only major differences observed for this strain compared to its parent strain.

For the porin-OprF-deficient mutant, strain H636 (Fig. 4), fewer siderophores were able to promote iron incorporation as compared to the other strains. Cepabactin and pyoverdine_{PAO} were the most efficient siderophores as for strain PAO1, and citrate, salicylic acid and pyochelin still promoted uptake at about 30% of the efficiency of cepabactin and pyoverdine_{PAO}. However, with the exception of coprogen, which still demonstrated a weak capacity to promote iron incorporation, none of the other siderophores promoted iron uptake in strain H636. This last group included desferriferrirobin, entero-

Fig. 1

Fig. 2

Fig. 1. ^{59}Fe uptake mediated by various iron ligands in *P. aeruginosa* PAO1 (ATCC 15692). The ligands are abbreviated as follows: cep, cepabactin; pvd, pyoverdine_{PAO}; cit, citrate; sal, salicylic acid; fch, desferriferriochrysin, pch, pyochelin, dfB, desferriferrioxamine B; fcr, desferriferrirocacin; cop, coprogen; dfE, desferriferrioxamine E. The line designated 'X' represents incorporation of ^{59}Fe liganded by desferriferriochrome A, enterobactin or desferriferriurubin.

Fig. 2. ^{59}Fe uptake mediated by various iron ligands in the pyoverdine-less mutant *P. aeruginosa* PAO6606. The ligands are abbreviated as in Fig. 1. The line designated 'X' represents incorporation of ^{59}Fe liganded by desferriferriochrome A, enterobactin or desferriferriurubin.

Fig. 3

Fig. 4

Fig. 3. ^{59}Fe uptake mediated by various iron ligands in the porin OprD-deficient mutant *P. aeruginosa* H673. Abbreviations of the ligands are the same as in legend of Fig. 1. The line designated 'X' represents incorporation of ^{59}Fe liganded by desferriferriochrome A, enterobactin, desferriferriurubin or desferriferrioxamine E.

Fig. 4. ^{59}Fe uptake mediated by various iron ligands in the porin-OprF-deficient mutant *P. aeruginosa* H636. The ligands are abbreviated as in Fig. 1. The line designated 'X' represents incorporation of ^{59}Fe liganded by desferriferriochrome A, enterobactin, desferriferriurubin, desferriferrioxamine E, desferriferrioxamine B, desferriferrirocacin or desferriferriochrysin.

bactin and desferriferriochrome A, as for the other strains, but also desferriferrioxamine B, desferriferrioxamine E, desferriferrirococin and desferriferriochrysin.

Discussion

From ^{59}Fe uptake studies involving 13 iron ligands mainly of microbial origin (siderophores) it can be concluded that *P. aeruginosa* ATCC 15692 (strain PAO1) is able to use the majority of the siderophores as an iron source. The only ones that were apparently not used by this bacterium were ferrichrome A and ferrirubin, two hydroxamate siderophores of fungal origin (van der Helm *et al.*, 1987; Konetschny-Rapp *et al.*, 1988), and enterobactin, the tricatecholate siderophore produced by several enterobacterial species, such as *E. coli* (O'Brien & Gibson, 1970), *Salmonella* (Pollack & Neilands, 1970) and *Shigella* (Payne, 1980).

Comparison of the uptake results with the growth stimulation properties of the siderophores towards the *P. aeruginosa* strains as visualized in plate bioassays (Table 1), indicated some discrepancies. For most of the siderophores, i.e. desferriferrirococin, desferriferriochrysin, desferriferrioxamine B, desferriferrioxamine E and pyoverdine_{PAO}, both methods resulted in the same conclusion — that the compounds had a stimulatory effect. Moreover, desferriferriochrome A and the pyoverdines from *P. aeruginosa* ATCC 27853, *P. fluorescens* ATCC 17400 and *P. fluorescens* CCM 2798, which had already been seen to be inefficient in promoting iron uptake in *P. aeruginosa* PAO1 (Hohnadel & Meyer, 1988; Cornelis *et al.*, 1989), gave consistent results with both methods. Discrepancies, however, were found for desferriferriubin and enterobactin, which promoted growth of all the strains tested, but were unable to promote iron uptake in these strains. In contrast, pyochelin and cepabactin, both efficient iron transporters as revealed by uptake assays, failed to promote bacterial growth in EDDHA-containing plate bioassays. Such results are not surprising considering the strong inhibitory effect of EDDHA on growth of exclusively cepabactin- and pyochelin-producing strains like PAO6606 (Hohnadel *et al.*, 1986) or *P. cepacia* ATCC 25416 (Meyer *et al.*, 1989), since these siderophores have a weaker affinity for iron than EDDHA.

As already discussed elsewhere (Hohnadel & Meyer, 1988), results of plate cross-feeding experiments always need to be interpreted with caution since uncontrolled phenomena like competition for iron between the added siderophore and EDDHA, or with the endogenous siderophore excreted by the bacteria during growth, or toxicity of a given siderophore, may result in artifactual

conclusions. Such undesired competition appears unlikely during uptake assays, done over a short period of time (15 min). Moreover, internal controls like the uptake results with strain PAO6606, a pyoverdine-deficient mutant, as well as the specific lack of iron incorporation with some siderophores for strain H636, attest to negligible iron exchange between siderophores during these assays.

Plate bioassays are still interesting, however, since they can reveal unsuspected features of siderophores, such as induction of specific iron transport pathways. K. Poole and colleagues have recently described the induction of an enterobactin-mediated iron uptake system by enterobactin itself in *P. aeruginosa* PAO1 (Poole *et al.*, 1990). This inducibility of enterobactin may explain the discrepancy between the cross-feeding and uptake assays observed with this compound. In plate bioassays the growing bacteria were in contact with the inducer for a long period of time (18 to 24 h), resulting in a growth stimulation effect likely to be due to induction of the enterobactin-mediated iron incorporation system, whereas this system was not induced during the uptake assays, since enterobactin and the non-proliferating cells were in contact for only a short period of time (15 min). It remains to be determined whether the desferriferriubin-related discrepancy results from such an induction, or is due to an iron exchange phenomenon.

The siderophores that promoted iron uptake by *P. aeruginosa* are of both bacterial and fungal origins, and belong to several different structural families. The fungal siderophores desferriferrirococin and desferriferriochrysin are structurally closely related to the inefficient siderophores desferriferriochrome A and desferriferriubin (van der Helm *et al.*, 1987; Konetschny-Rapp *et al.*, 1988). Coprogen is a fungal trihydroxamate siderophore made from three δN -hydroxy- δN -acylornithine units (van der Helm *et al.*, 1987). In addition, the present study included two desferriferrioxamine compounds of bacterial origin, desferriferrioxamine B produced by actinomycetes (Bickel *et al.*, 1960), and desferriferrioxamine E, produced by actinomycetes and also by some other bacterial species, such as *Erwinia herbicola* (Berner *et al.*, 1988) and *Pseudomonas stutzeri* (Meyer & Abdallah, 1980). The other effective siderophores are structurally unrelated to the siderophores cited above and included cepabactin, a pyridinone compound produced by the non-fluorescent *Pseudomonas cepacia* ATCC 25416 (Meyer *et al.*, 1989), and salicylate, which is a precursor of pyochelin (Ankenbauer & Cox, 1988), but also acts as a siderophore for a strain of *Pseudomonas fluorescens* (unpublished results). Together with these exogenous siderophores and the siderophores pyochelin and pyoverdine, produced by *P. aeruginosa* PAO1 itself (Cox & Graham, 1979; Cox & Adams, 1985), citrate was also able to mediate iron

incorporation in this strain, a result in agreement with previous findings (Cox, 1980; Harding & Royt, 1990).

Among the 10 iron-chelating compounds able to promote iron uptake in *P. aeruginosa* PAO1, two of them, pyoverdine_{PAO} and cepabactin, were particularly efficient compared with the others. For the wild-type strain PAO1, as well as for the two insertion mutants, H636 and H673, with altered porins, these two compounds led to an incorporation of iron that was two to three times greater than that observed with the next most efficient iron chelator, citrate.

It is not surprising that pyoverdine_{PAO} was one of the most effective siderophores in promoting iron uptake since it is the endogenous siderophore of *P. aeruginosa* PAO1. The iron uptake efficiency for the second endogenous siderophore, pyochelin, was considerably lower, representing for strains PAO1, H636 and H673 less than 30% of the iron incorporation observed with pyoverdine. Such results support the conclusion that pyoverdine is the major siderophore of *P. aeruginosa* PAO1, in agreement with results on the comparative pathogenicity of pyoverdine-less or pyochelin-less mutants of *P. aeruginosa* PAO1 (Ankenbauer *et al.*, 1985). However, the present study demonstrates that, under appropriate circumstances, pyochelin can be more efficient than pyoverdine, as shown for the pyoverdine-less mutant PAO6606. After 15 min incubation this strain had incorporated twice as much radioactivity as the other strains when iron was chelated by cepabactin, salicylate or citrate, whereas pyochelin-mediated iron incorporation was 4.5 times greater in this strain compared to pyochelin-mediated incorporation in the other strains. In contrast, using pyoverdine, iron was taken up with similar efficiency by all strains, including PAO6606. Therefore, in PAO6606, pyochelin supported nearly 2-fold higher iron uptake than pyoverdine. From these results it seems that inability to synthesize pyoverdine results, in response to iron starvation, in increased efficiency of only some of the other possible iron acquisition routes, eg. cepabactin- or pyochelin-mediated iron uptake. Previous studies concerning the phenotypic characterization of the mutant PAO6606 (Hohnadel *et al.*, 1986) demonstrated that this mutation, affecting the biosynthesis of pyoverdine, had no apparent effect on the outer membrane proteins of this bacterium. Thus, the mechanism by which *P. aeruginosa* PAO6606 is able to increase the efficiency of its secondary iron uptake systems is apparently not related to an overexpression of IROMPs.

Among the siderophores which are not produced by *P. aeruginosa* but were effective in iron incorporation, cepabactin, as mentioned above, demonstrated an effectiveness equivalent to that of the major endogenous siderophore of *P. aeruginosa*. Salicylate and citrate,

which can be considered as siderophore-like compounds, were two other compounds which, regardless of the strain tested, resulted in a lesser but nevertheless consistent iron uptake efficiency. Cepabactin, 1-hydroxy-5-methoxy-6-methyl-2(1H)-pyridinone (Meyer *et al.*, 1989), has no chemical relationship with pyoverdine (Briskot *et al.*, 1989) or pyochelin (Cox *et al.*, 1981). Therefore, it appears likely that a special receptor may be involved at the level of the *P. aeruginosa* outer membrane for recognition of the 3:1 cepabactin-iron complex.

Under iron-starvation, *P. aeruginosa* strains usually express several IROMPs (Cornelis *et al.*, 1987, 1989), the major one, a protein with an apparent molecular mass of 80 kDa being the receptor for the pyoverdine-iron complex in strain PAO1 (Meyer *et al.*, 1990). A search for mutants affected in the other IROMPs should clarify which protein could serve as the ferric cepabactin receptor in the outer membrane of *P. aeruginosa* PAO1. A ferric-citrate receptor, corresponding to a 43 kDa outer-membrane protein, has recently been postulated in *P. aeruginosa* PAO1 (Harding & Royt, 1990), suggesting the existence of a specific route for iron incorporation through this compound, similar to the FecA function in *E. coli* (Wagegg & Braun, 1981). The receptor, if any, for iron-salicylate remains to be determined. Since this compound is a precursor and part of the structure of pyochelin, one could postulate that the 14 kDa and/or the 75 kDa IROMPs of *P. aeruginosa* may act as receptor(s) for both pyochelin- (Sokol & Woods, 1983; Heinrichs *et al.*, 1991) and salicylate-iron complexes.

The remaining exogenous siderophores able to promote iron uptake in *P. aeruginosa*, desferriferrioxamine B, desferriferrioxamine E, desferriferriochrysin and desferriferriicrocin, generally had a substantially lower efficiency compared to the other siderophores and, interestingly, were totally inefficient when tested with the strain H636. This strain has an insertion in the gene for the outer-membrane protein OprF (Woodruff & Hancock, 1988), which has been shown to function as a porin, being a major uptake route across the outer membrane for hydrophilic compounds having a molecular mass less than 3000 Da (Nikaido & Hancock, 1986). This would suggest that ferrisiderophores may penetrate the bacterial cell through this pathway, and that the porin OprF is the only uptake route for iron complexes of desferriferrioxamine B, desferriferrioxamine E, desferriferriochrysin and desferriferriicrocin. However, why ferriochrome A, ferrirubin and ferrienterobactin, which were shown to be inefficient for all the OprF⁺ strains, were unable to use this route, remains to be explained.

The siderophores taken up by specific pathways involving IROMPs like pyoverdine or pyochelin in *P. aeruginosa* may also penetrate through porin channels.

K. Poole and colleagues have recently isolated a *P. aeruginosa* mutant lacking the ferripyoverdine receptor (Poole *et al.*, 1991). Interestingly, this mutant still presented a very low level of pyoverdine-mediated iron uptake, in a range of efficiency similar to that observed in the present study for desferriferrioxamine-, desferriferri-chrysin- and desferriferrirocine-mediated iron uptake. The authors suggested the presence of a second uptake system for ferripyoverdine in *P. aeruginosa* which, in the light of results presented here, may be the porin pathway. More direct experimental evidence, such as permeability studies involving ferrisiderophores and porin-OprF proteoliposomes, are needed to assess this route of penetration for iron in *P. aeruginosa*.

In conclusion, this study underlines the particularly important role of iron in cellular metabolism by demonstrating that, beside the two endogenous systems involving pyoverdine and pyochelin and their respective receptors, *P. aeruginosa* is also able to fulfill its iron requirement by using exogenous siderophores and exogenous-siderophore-mediated iron uptake pathways, among them the porin-OprF channels. Another important feature revealed in this study is the increased capacity of iron uptake through secondary systems when the major route of iron incorporation becomes deficient. This unexpected mechanism of regulation remains to be explored.

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