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SHORT COMMUNICATION

Susceptibility of *Pseudomonas aeruginosa* to catechol-substituted cephalosporin is unrelated to the pyochelin–Fe transporter FptA

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Abstract Previously it has been postulated that the pyochelin–Fe outer membrane transporter, FptA, is involved in the uptake of catechol-substituted cephalosporins in *Pseudomonas aeruginosa*. Iron uptake and antibacterial activity studies on different mutants showed clearly that FptA is unable to bind and transport these antibiotics.

Keywords Siderophore · Iron uptake · Cephalosporine · Outer membrane transporter · TonB

Under iron-limiting growth conditions, aerobic bacteria produce siderophores, which form complexes with Fe^{3+} in the environment and deliver it, via specific membrane transporters, to the bacterial cytoplasm. The two major iron transport systems characterized in *Pseudomonas aeruginosa* under iron-limiting growth conditions, involve the siderophores pyoverdine (PVD) and pyochelin (PCH) and their corresponding outer membrane transporters, respectively, FpvA and FptA (for a review see Schalk 2008). PVD is composed of a dihydroxyquinoline chromophore to which, an octapeptide chain is attached and the structure of PCH is represented in Fig. 1. The stoichiometry of Fe(III) chelation by this siderophore can be 2:1 or 1:1 (PCH:Fe(III)), depending on the PCH:Fe ratio and the pH of the solution (Tseng et al. 2006).

β-Lactam antibiotics that possess a catechol or pyridone moiety have been reported to show potent antibacterial activities against most gram-negative bacteria, including *P. aeruginosa*. It was proposed that this enhanced activity was due to the ability to chelate iron and to undergo active transport into cells by iron transport pathways. The outer membrane receptors involved in this catechol-substituted cephalosporin transport were shown, in *Escherichia coli*, to be the siderophore outer membrane transporters Cir and Fiu (Curtis et al. 1988; Nikaido and Rosenberg 1990; Tatsumi et al. 1995), suggesting that these two transporters have a quite broad binding selectivity for catechol analogues. Concerning *P. aeruginosa*, Gensberg et al. (1994) suggested that BRL 41897A, a C α-formamido substituted cephalosporin (Fig. 1), was transported into this bacteria via the PCH–Fe transport system. The studies reported a strong correlation between sensitivity to these molecules and expression of FptA, the PCH outer membrane transporter. However, no mutant with an FptA deletion was used, only growth conditions (presence or absence of iron or of PCH) allowing an increase or a decrease of FptA expression. This possibility of BRL 41897A to be transported via FptA appeared contradictory with the high siderophore selectivity and stereospecificity of FptA described previously by our group: only PCH is able to bind to FptA (Cobessi et al. 2005; Mislin et al. 2006), even the PCH enantiomere (Youard et al. 2007; Hoegy et al. 2009) is unable to be transported by this protein. Therefore, we found important to investigate the ability of FptA to interact with BRL 41897A and its analogue BRL 42948A (Fig. 1). The ability of BRL 41897A and BRL 42948A to

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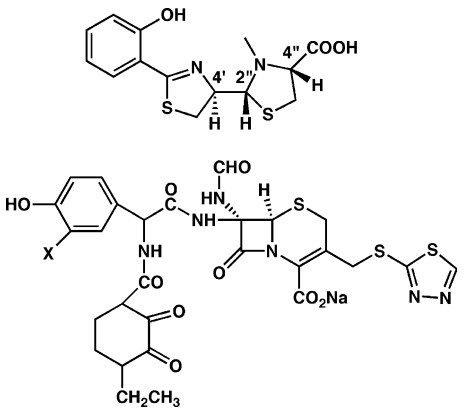

Fig. 1 Structure of PCH (*upper* molecule) and BRL 41897A, X = OH; BRL 42948A, X = H (*lower* molecule) (Critchley et al. 1991)

transport ^{55}Fe was carried out as described previously for PCH and PVD (Schalk et al. 2001; Mislin et al. 2006). The cells were grown overnight in iron deficient conditions (succinate medium (Demange et al. 1990)), harvested, washed and resuspended in 50 mM Tris HCl pH 8.0. When PAO1 cells were used, ^{55}Fe was taken up with comparable efficiency in the presence of PCH, BRL 41897A and BRL 42948A (Fig. 2a). Surprisingly, when this experiment was repeated with a *P. aeruginosa* strain unable to produce either PCH or PVD (PAD07, Takase et al. 2000a), no iron uptake was observed (Fig. 2b), suggesting that the uptake observed with PAO1 was due to the presence of PCH and/or PVD, produced by the bacteria, adsorbed at the bacterial cell surface and not removed by the washing step. In order to confirm this hypothesis, the experiment was repeated

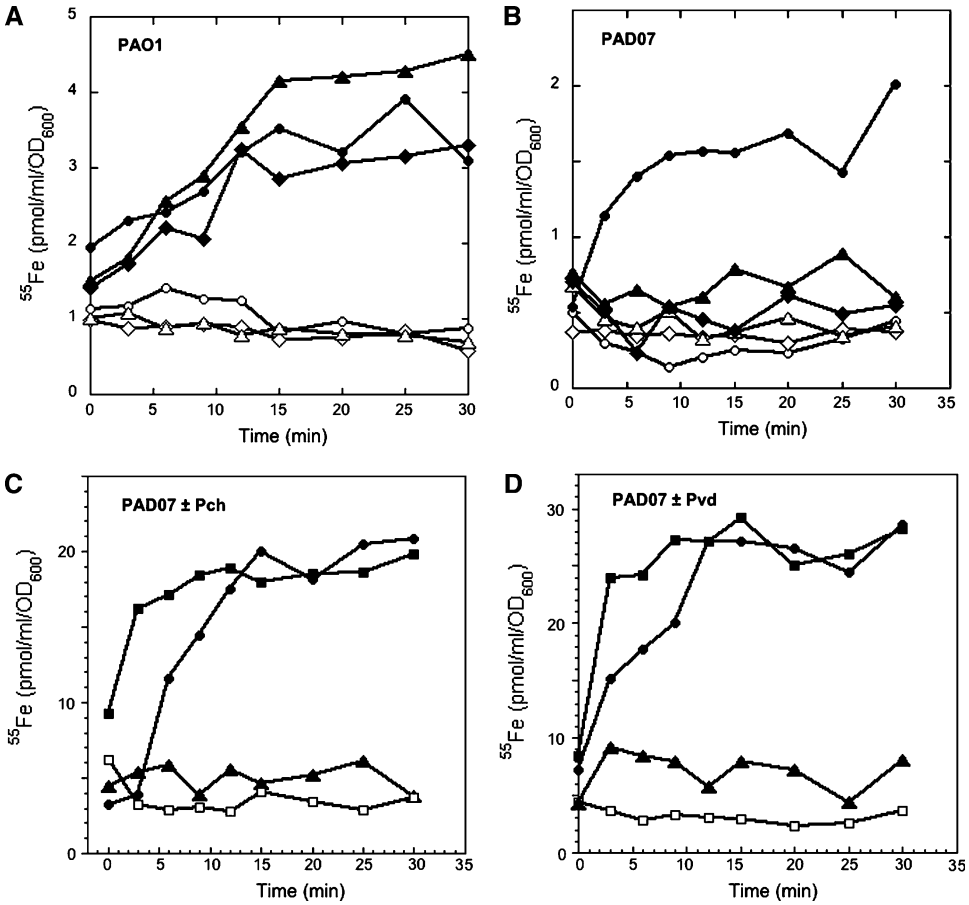

Fig. 2 ^{55}Fe uptake in *P. aeruginosa* PAO1 in graph **a**, in the PCH- and PVD-deficient PAD07 strain in graphs **b**–**d**. For graph **a** and **b**, PCH (*filled circle*), BRL 41897A (*filled triangle*) and BRL 42948A (*filled diamond*) were loaded with ^{55}Fe and at a concentration of 10 nM mixed with the cells at an OD_{600} of 1. The iron uptake kinetics were repeated in the presence of 200 μM of the protonophore CCCP (*open circle* for PCH–^{55}Fe, Δ for BRL 41897A–^{55}Fe and *open diamond* for BRL 42948A–^{55}Fe). For graph **c**, PCH–^{55}Fe (*filled circle*) and BRL 41897A–^{55}Fe (*filled triangle*) at 100 nM were mixed with PAD07 cells. The experiment was repeated for BRL 41897A–^{55}Fe, in the presence of 200 nM PCH (*filled square*) or 200 nM PCH and 200 μM CCCP (*open square*). For graph **d**, PVD–^{55}Fe (*filled circle*) and BRL 41897A–^{55}Fe (*filled triangle*) at 100 nM were mixed with PAD07 cells. The experiment was repeated for BRL 41897A–^{55}Fe, in the presence of 200 nM PVD (*filled square*) or 200 nM PVD and 200 μM CCCP (*open square*)

Table 1 Effect of secretion of PVD, PCH and mutation of FptA on the antibiotic activity of BRL 41897A

Strains used with phenotypes	Diameter of zone of growth inhibition (cm)	
	1 mM BRL 41897A	10 mM BRL 41897A
PAO1	1.77 ± 0.15	2.50 ± 0.10
PAD07 (ΔPch, ΔPvd; (Takase et al. 2000b))	1.83 ± 0.06	2.63 ± 0.17
K2388 (deletion of *fptA* (Mislin et al. 2006))	1.57 ± 0.21	2.63 ± 0.15

This study was carried out only with the form having the catechol function. BRL 41897A was spotted onto filter paper disks (diameter of 0.5 cm) and applied to the succinate medium agar plates. The plates were incubated at 37°C for 20 h and the diameter of the zone of growth inhibition measured

with PAD07, BRL 41897A–^{55}Fe combined with either metal-free PCH (Fig. 2c) or PVD (Fig. 2d), and ^{55}Fe uptake occurred again as in PAO1 (data not shown for BRL 42948A). All these data clearly showed that BRL 41897A and BRL 42948A are unable to transport ^{55}Fe into *P. aeruginosa*. When, PAD07 cells were incubated in the presence of 1 nM PCH–^{55}Fe and increasing concentration of BRL 42948A (0.001–10 μM), no competition between both molecules for the FptA binding site was observed (data not shown). In order to have only a competition between the molecules for the FptA binding site, this experiment was carried out only with BRL 42948A, the form having no catechol function and being unable to compete efficiently with PCH for ^{55}Fe chelation. Finally, the antibiotic activity of these molecules was not abolished or diminished by deletion of FptA (Table 1), indicating again that catechol-substituted cephalosporins are not transported into *P. aeruginosa* by the PCH pathway. These results confirm again the high binding selectivity of FptA and the ferri-PCH uptake pathway. Above all shows that this outer membrane protein is unable to transport catechol-substituted cephalosporins in *P. aeruginosa*. This raises the question as to the uptake mechanism for catechol-substituted cephalosporins in *P. aeruginosa,* and the siderophore outer membrane transporters Cir and Fiu (transporters of dihydroxybenzylserine) merit further study in this context.

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