

Iron acquisition from transferrin and lactoferrin by *Pseudomonas aeruginosa* pyoverdinin

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Growth of *Pseudomonas aeruginosa* ATCC 15692 was promoted when the strain was cultured in an iron-depleted succinate medium, supplemented with transferrin at 30%, 60% and 100% and lactoferrin at 60% and 100% iron-saturation. No significant differences between cell growth and pyoverdinin production were observed when transferrin iron saturation was increased from 30% to 100%; however, cell growth and pyoverdinin production were strongly dependent on lactoferrin iron saturation. Lower lactoferrin iron saturation (< 30%) resulted in more pyoverdinin production and reduced cell growth. Incubation of pyoverdinin (1.0 µM) with 10.0 µM transferrin (30%, 60% and 100% iron-saturated) or lactoferrin (60% and 100% iron-saturated) led to quenching of pyoverdinin fluorescence. Also, 24 h incubation of pyoverdinin (20.0 µM) with these two proteins (20.0 µM, 30%, 60% and 100% iron-saturated transferrin and 60% and 100% iron-saturated lactoferrin) at 25 °C resulted in increased absorbance at 460 nm. Both the fluorescence quenching and absorbance increases were iron-saturation-dependent. Taken together, these results support the conclusion that at physiological pH, *P. aeruginosa* pyoverdinin can acquire iron from partially iron-saturated transferrin or lactoferrin.

Keywords: *Pseudomonas aeruginosa*, transferrin, lactoferrin, pyoverdinin

INTRODUCTION

Pseudomonas aeruginosa is an opportunistic pathogen which is frequently found in clinical specimens from burns, surface wounds, urinary tract, ear and eye infections, and is commonly isolated from the lungs of patients with cystic fibrosis (CF) (Doggett *et al.*, 1966; Reynolds *et al.*, 1976). In response to iron deprivation *P. aeruginosa* produces two unrelated siderophores, pyoverdinin and pyochelin, as well as membrane receptors for binding the corresponding iron–siderophore complexes. Both of these siderophores promoted the growth of *P. aeruginosa* when added to medium with iron–transferrin or human sera as the iron sources (Ankenbauer *et al.*, 1985). Because of the higher iron–pyoverdinin binding constant (10^{32}) (Demange *et al.*, 1990), in comparison to that of iron–pyochelin (10^5) (Cox & Graham, 1979), pyoverdinin was considered to be more effective. It has been reported that a pyochelin-deficient mutant (Pvd⁺ Pch⁻) strain grew as well as the parent strain whereas a pyoverdinin-deficient mutant (Pvd⁻ Pch⁺) exhi-

bited severely retarded growth (Ankenbauer *et al.*, 1985).

Although pyoverdinin has been shown to stimulate growth of *P. aeruginosa* in iron–transferrin containing medium (Ankenbauer *et al.*, 1985) and to restore the growth of pyoverdinin-deficient mutants in bicarbonate-apotransferrin-containing succinate medium (Meyer *et al.*, 1996), the role of pyoverdinin in *P. aeruginosa* infections warrants further study. For example, using the membrane dialysis technique, Sriyosachati & Cox (1986) found that ⁵⁵Fe acquisition from transferrin by pyoverdinin was observed only in the presence of pyoverdinin and live *P. aeruginosa* cells at physiological pH 7.4 or at pH values between 5.0 and 6.0 without living cells. However, Döring *et al.* (1988) showed that pyoverdinin was unable to acquire iron from transferrin at physiological pH, and that rapid iron release and pyoverdinin iron uptake from transferrin were only observed in the presence of *P. aeruginosa* elastase. Wolz *et al.* (1994) have reported that incubation of iron–transferrin (100% iron saturation) with purified pyoverdinin in concentrations similar to those found in the culture supernatant (40 µM Fe₂–transferrin, 40 µM

Abbreviations: CF, cystic fibrosis; ICP, inductively coupled plasma.

pyoverdin) resulted in release of iron from transferrin after 10 h at 37 °C. The discrepancy between this and their previous results was attributed to differences in the pyoverdin/transferrin ratio (Wolz *et al.*, 1994). More recently, Meyer *et al.* (1996) have reported that when fully iron-saturated [⁵⁹Fe]transferrin (0.5 µM) and pyoverdin (4–8 µM) were mixed in an *in vitro* assay, the maximal amount of radioactivity removed from ferri-transferrin was reached very rapidly. Also, it has been reported by Haas *et al.* (1991a) that all sputum samples from the lungs of CF patients harbouring *P. aeruginosa* infections contained pyoverdin with a degree of ferration in excess of 50%. Furthermore, like laboratory and clinical non-mucoid, *P. aeruginosa* isolates (Cornelis *et al.*, 1989; Cox & Graham, 1979; Wendenbaum *et al.*, 1983), six mucoid *P. aeruginosa* strains isolated from CF patients synthesized pyoverdin when grown in iron-deficient media (Haas *et al.*, 1991b). Despite these findings, the role of pyoverdin in *P. aeruginosa* infection has not been fully elucidated especially with partially iron-saturated proteins.

Iron-binding proteins, transferrin and lactoferrin, are important in host defence (Aisen & Listowsky, 1980; Weinberg, 1974). The presence of unsaturated iron-binding proteins has been shown to contribute to the bacteriostatic and bactericidal activity of serum and secretions. Saturation of these proteins with iron abolished the inhibitory effects and allowed the growth of micro-organisms (Bullen & Armstrong, 1979; Finkelstein *et al.*, 1983; Lankford, 1973). Therefore, the ability of siderophores to acquire iron from these proteins, or the direct utilization of iron bound to these proteins by bacteria, are essential for the maintenance of prokaryotic infections in the host. Thus, iron saturation of these proteins is important for such iron acquisition. Wolz *et al.* (1994) found that iron acquisition by pyoverdin from 100% iron-saturated transferrin (40 µM) was only possible at high pyoverdin concentrations (40 µM). Also, Meyer *et al.* (1996) reported that pyoverdin (4–8 µM) can acquire iron from fully iron-saturated transferrin. Yet in the human bloodstream iron–transferrin saturation is much lower than 100%. For example, only about one-third of the transferrin iron-binding sites were reported to be occupied by iron (Bezkorovainy, 1987). In addition, pyoverdin concentrations were reported to be in the range 0.48–1.55 µM in CF sputum samples (Haas *et al.*, 1991a, b). Given the above findings, it is important to determine (Aisen & Listowsky, 1980) whether *P. aeruginosa* can acquire and use iron for growth from partially iron-saturated transferrin and if so (Ankenbauer *et al.*, 1985), whether this iron-acquisition process can be demonstrated *in vitro* with purified pyoverdin.

In this study, *P. aeruginosa* PAO1 (ATCC 15692) was cultured in an iron-depleted medium, supplemented with various quantities of iron-saturated transferrin and lactoferrin (0, 30, 60 and 100%). Cell growth was stimulated in all cases except in the apo-transferrin, apo-lactoferrin and the 30% iron-saturated lactoferrin-containing medium. Lower iron saturation in lactoferrin

promoted more pyoverdin production and reduced cell growth. At physiological pH, incubation of purified *P. aeruginosa* pyoverdin with 30%, 60% and 100% iron-saturated transferrin or lactoferrin resulted in an iron-saturation-dependent fluorescence quenching and absorbance enhancement at 460 nm. These results strongly suggest that pyoverdin can acquire iron over a wide range of transferrin iron saturations (30–100%) and a higher range of lactoferrin iron saturations (60–100%).

METHODS

Micro-organism and pyoverdin production. *Pseudomonas aeruginosa* PAO1 (ATCC 15692) was obtained from the American Type Culture Collection Rockville, MD, USA. A synthetic succinate medium was used for pyoverdin production as described previously (Xiao & Kisaalita, 1995). Incubation was carried out at 37 °C and 200 r.p.m. in a New Brunswick Innova 4000 shaker/incubator for 15 h. Cells were removed by centrifugation (10000 g for 10 min at 4 °C). The supernatant was further membrane-filtered (0.25 µm, Amicon) to yield a cell-free solution of crude pyoverdin. Pyoverdin production was estimated by measuring A_{400} of the supernatant with a Beckman DU 650 spectrophotometer.

Purification of pyoverdins. Pyoverdins were purified by a modified procedure, previously described by Xiao & Kisaalita (1995). Briefly, the *P. aeruginosa* PAO1 cell-free supernatant buffered with 1 M HEPES (pH 7.0) was applied to a Chelating Sepharose Fast Flow column (1.5 × 25 cm, Pharmacia LKB) pre-saturated with CuSO₄ and equilibrated with 20 mM HEPES (pH 7.0) containing 100 mM NaCl at a flow rate of 100 ml h⁻¹. The column was washed with 20 mM HEPES and eluted with 20 mM sodium acetate buffer (pH 6.0 and 5.0) containing 100 mM NaCl. Fractions corresponding to four peaks (detected on the basis of their A_{400}) were separately pooled and lyophilized. The dried material was dissolved in 1 ml distilled water containing 10 mM EDTA and then applied to a Sephadex G-15 column (1.5 × 100 cm), pre-equilibrated with deionized water. The elution was carried out with distilled water at a flow rate of 20 ml h⁻¹. Fractions (3 ml) were collected and their A_{400} was measured. Fluorescence was measured with a Perkin Elmer Fluorometer (LS-50) at emission and excitation wavelengths of 460 and 400 nm, respectively. The pH of the samples for fluorescence measurements was adjusted to 7.0 prior to analysis. Those fractions containing pyoverdin were pooled, lyophilized, and stored at 4 °C until needed. Of the four PAO1 pyoverdins purified (Pa-A, Pa-B, Pa-C and Pa-D), the major pyoverdin, Pa-C was used in this study.

Preparation of iron-binding proteins. Human transferrin and lactoferrin were obtained in iron-poor form (Sigma). Iron-free proteins were prepared by dialysing proteins against 0.1 M sodium citrate buffer. The dialysis process was performed at pH 4.5 for transferrin and pH 2.0 for lactoferrin. The proteins were diluted in a solution of 40 mM Tris/20 mM sodium bicarbonate buffer (pH 7.4) to give a final protein concentration of 200 µM. The proteins were 30%, 60% and 100% saturated with iron by addition of 120, 240 and 400 µM ferric chloride and then incubated at 37 °C for 1 h. Unbound iron was removed by overnight dialysis using Slide-a-Lyzer dialysis cassette with a molecular mass cut-off of 10000 Da (Pierce) against 1 litre 40 mM Tris/20 mM sodium bicarbonate buffer (pH 7.4) with two changes. The proteins were filter-sterilized and stored at 4 °C until needed. The protein concentrations were determined by the micro-method of Bradford (1976) with the Coomassie protein assay reagent (Pierce) and with bovine

serum albumin as the standard. The iron concentration was determined by the ferrozine method (Stookey, 1970) (Sigma procedure no. 565). Free iron contamination was checked by the inductively coupled plasma (ICP) spectroscopic technique after filtering the samples with micro-concentrators (Centricon; 10000 Da cutoff, Amicon).

Cell growth and pyoverdinin production assay. A synthetic succinate medium was used (Xiao & Kisaalita, 1995). The iron concentration of the medium was determined by the ICP atomic emission spectroscopic technique at the Chemical Analysis Laboratory of the University of Georgia. Apo-transferrin, apo-lactoferrin or 30%, 60%, and 100% iron-saturated transferrin and lactoferrin were added to the medium to a final concentration of 1.25 μM . Free iron was added from a 25 mM FeSO_4 solution which had been freshly prepared and sterile-filtered. Bacteria from slants served as inocula for 25 ml preculture in 125 ml flasks. Cells were harvested by centrifugation and washed once with a sterilized succinate medium prior to use as inocula. Inoculation were performed with an initial OD_{600} value of 0.035. Incubation was carried out at 37 °C and 200 r.p.m. in a New Brunswick Innova 4000 shaker/incubator. Cell growth was estimated by measuring OD_{600} . The amount of pyoverdinin produced was estimated by determining the A_{400} of the supernatant after adjusting the pH to 7.0 with 0.1 M Tris/HCl buffer. The fluorescence of the supernatant was also determined at emission and excitation wavelength of 460 and 400 nm, respectively.

Fluorimetric and spectrometric assay. Two methods were employed for the determination of pyoverdinin iron acquisition from 30%, 60% and 100% iron-saturated transferrin and lactoferrin. These methods are based on the fact that pyoverdinin fluorescence quenching and increase in A_{460} are indicators of Fe^{3+} -pyoverdinin complex formation (Xiao & Kisaalita, 1995).

(i) **Fluorimetric method.** This technique permits continuous monitoring of iron acquisition from iron-binding proteins by direct use of pyoverdinin. Portions (2 ml) of purified pyoverdinin solution [1.0 μM in 0.1 M HEPES buffer (pH 7.4)] were incubated at 25 °C with stirring, and iron release from 30%, 60% and 100% iron-saturated transferrin and lactoferrin was initiated by adding these protein solutions to a final concentration of 10.0 μM . The fluorescence due to iron-pyoverdinin formation was monitored continuously for 10 min at emission and excitation wavelengths of 460 and 400 nm, respectively. Iron-free proteins were used as controls instead of iron-bound proteins.

(ii) **Spectrometric method.** Pyoverdinin (20.0 μM) was incubated with transferrin or lactoferrin (20.0 μM at 0%, 30%, 60% and 100% iron saturation) in 0.1 M HEPES buffer (pH 7.4) at 25 °C for 24 h. The absorption spectra were measured from 340 to 640 nm, after proteins had been removed from the reaction mixture by ultrafiltration (4000 r.p.m., 25 min, 4 °C) with micro-concentrators (Centricon; 10000 Da cutoff, Amicon). Iron-free proteins were used as controls instead of iron-bound proteins.

RESULTS

Growth of *P. aeruginosa* ATCC 15692 in medium containing apo-, 30%, 60% and 100% iron-saturated transferrin or lactoferrin

As shown in Fig. 1(a), apo-transferrin had no growth-promotion effect on *P. aeruginosa* ATCC 15692, while addition of 30%, 60% and 100% iron-saturated trans-

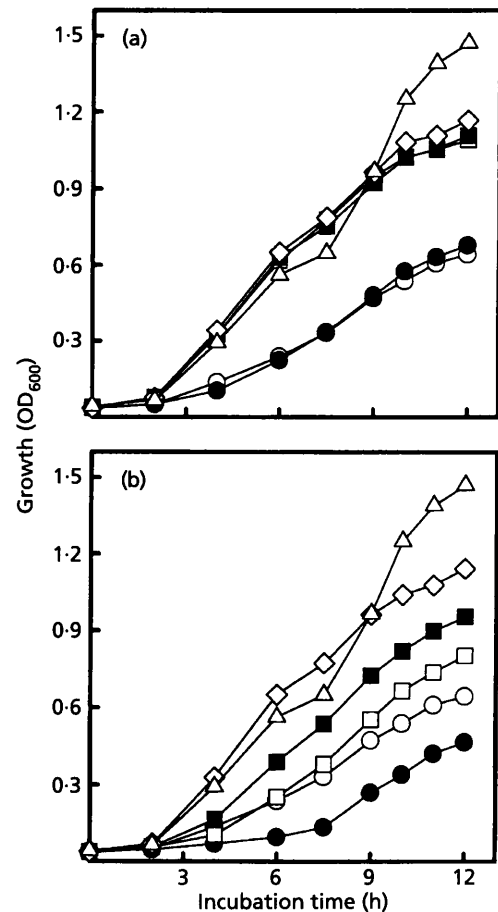


Fig. 1. Effect of iron saturation level of transferrin (a) and lactoferrin (b) on the growth of *P. aeruginosa* ATCC 15692. Cells were grown in a succinate medium containing 1.5 μM transferrin or lactoferrin. Washed bacterial cells were inoculated at a OD_{600} 0.035 without addition of iron (○), at 0% iron saturation (●), 30% iron saturation (□), 60% iron saturation (■), 100% iron saturation (◇) and with 50 μM iron added (Δ).

ferrin (1.25 μM) resulted in significant growth promotion similar to that due to addition of free iron (50 μM). Pyoverdinin production was estimated by measuring the absorption spectra of the supernatants after incubation for 12 h at 37 °C. *P. aeruginosa* ATCC 15692 produced almost identical amounts of pyoverdinin when grown in iron-free (no iron addition) and apo-transferrin supplemented media (Fig. 2a). Pyoverdinin production decreased significantly when cells were grown in the medium supplemented with 30%, 60% and 100% iron-saturated transferrin. This was consistent with iron-mediated down-regulation of pyoverdinin synthesis. Pyoverdinin was not produced when the medium was supplemented with 50 μM iron since no absorbance (A_{400}) was detected (Fig. 2a). Using both fluorimetric and spectrometric techniques, pyoverdinin production was found to be reduced to approximately 25% of control (no iron addition) by addition of 30%, 60% and 100% iron-saturated transferrin.

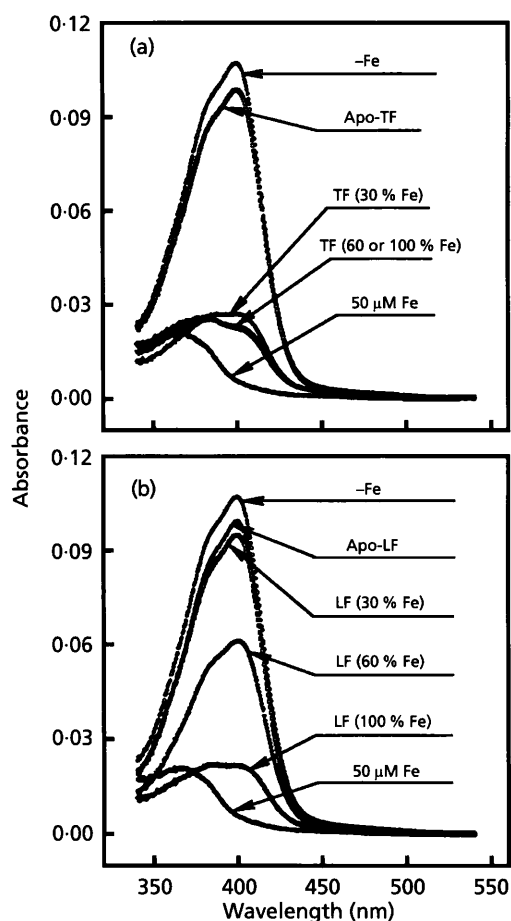


Fig. 2. Effect of iron saturation level of transferrin (TF) (a) and lactoferrin (LF) (b) on pyoverdinin production by *P. aeruginosa* ATCC 15692. The absorption spectra of the supernatants were measured after removal of cells by centrifugation (10000 g, 10 min) and adjusting the pH to 7.0 with 100 mM Tris/HCl buffer.

In contrast to the identical growth curves for iron-free or apo-transferrin-containing media, cells in apo-lactoferrin supplemented medium exhibited retarded growth in comparison to the iron-free medium (Fig. 1b). With lactoferrin, the relative extent of cell growth promotion was observed to correspond to the iron saturation level (Fig. 1b). Also, the absorption spectra of culture supernatants showed that pyoverdinin production by this strain was inversely related to the iron saturation level of lactoferrin (Fig. 2b). The close agreement between the spectrometric and fluorimetric methods as well as pyoverdinin production-dependence on iron saturation are evident in Fig. 3.

Iron acquisition from transferrin and lactoferrin by the purified *P. aeruginosa* pyoverdinin Pa-C

Based on the observation that transferrin and lactoferrin promoted growth of *P. aeruginosa*, we hypothesized that pyoverdinin and lactoferrin should be able to acquire iron directly from transferrin or lactoferrin. To confirm

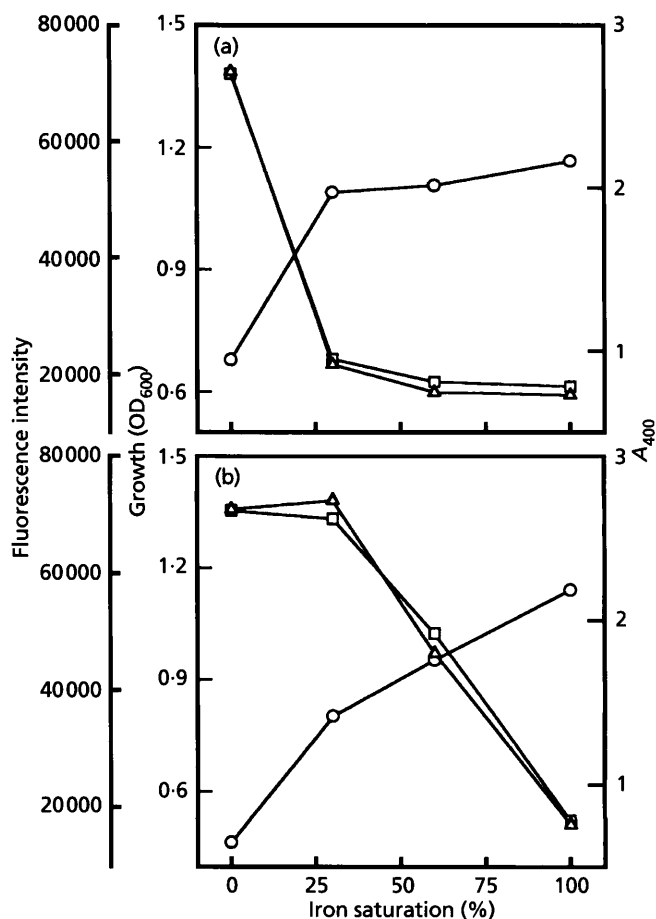


Fig. 3. Effect of iron saturation level of transferrin (a) and lactoferrin (b) on cell growth and pyoverdinin production by *P. aeruginosa* ATCC 15692. Cell growth was estimated by measuring OD₆₀₀. Pyoverdinin production was estimated by measuring both A₄₀₀ and fluorescence intensity (arbitrary units) (EM/EX = 460 nm/400 nm) at pH 7.0: OD₆₀₀ (○), A₄₀₀ (□) and fluorescence intensity (△).

this, two methods described in the Methods were employed for the determination of iron acquisition from 30%, 60% and 100% iron-saturated transferrin and lactoferrin by the main *P. aeruginosa* pyoverdinin, Pa-C. As shown in Fig. 4(a), at physiological pH, 13%, 27% and 39% of pyoverdinin (1.0 μM) fluorescence was quenched by addition of 30%, 60% and 100% iron-saturated transferrin (10.0 μM), respectively, after 10 min incubation at 25 °C. Using a pyoverdinin fluorescence change calibration curve (vs added free iron), the amount of iron removed from transferrin and lactoferrin was calculated and the results are presented in Table 1. To ascertain whether iron-pyoverdinin complexes were formed as suggested by the observed fluorescence quenching, pyoverdinin (20.0 μM) was incubated with transferrin (20.0 μM) at 25 °C for 24 h. Transferrin was removed from the reaction mixture by ultrafiltration and the presence of iron-pyoverdinin complexes was monitored by measuring the absorption spectrum from 340 to 640 nm. As shown in Fig. 5(a),

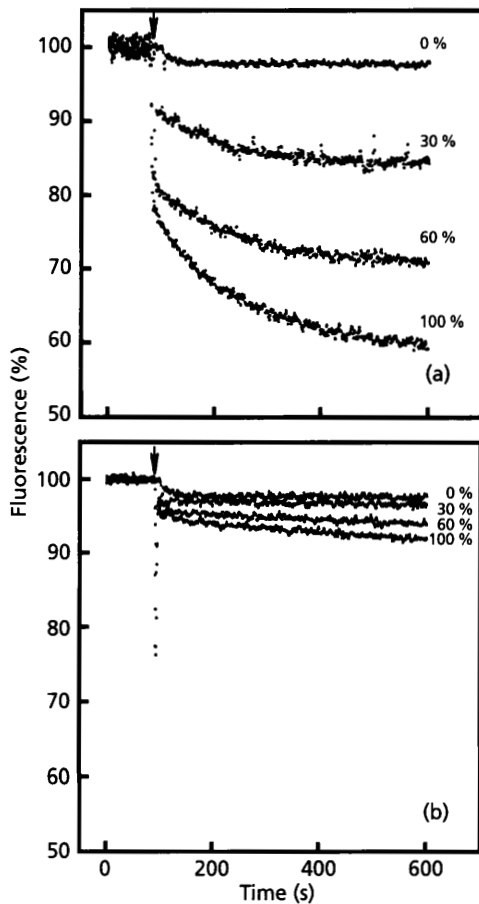


Fig. 4. Fluorescence quenching of *P. aeruginosa* pyoverdin Pa-C by transferrin (a) and lactoferrin (b) at physiological pH. Pyoverdin Pa-C was added to 2.0 ml 100 mM HEPES buffer (pH 7.4) to a final concentration of 1.0 μ M. Transferrin and lactoferrin with various iron saturation levels were added at the times indicated by the arrows to final concentrations of 10.0 μ M. Fluorescence was measured at an excitation wavelength of 400 nm and an emission wavelength of 460 nm. The slit width for excitation and emission was 5.0 nm. Fluorescence is expressed as a percentage of the initial fluorescence intensity.

Table 1. Iron(III) removal from transferrin and lactoferrin by *P. aeruginosa* ATCC 15692 pyoverdin Pa-C

Protein iron saturation (%)	Iron(III) (μ M)*
Transferrin	
30	0.104
60	0.218
100	0.347
Lactoferrin	
30	—
60	0.031
100	0.046

*Iron(III) was calculated from the fluorescence change after 10 min incubation (see Fig. 4).

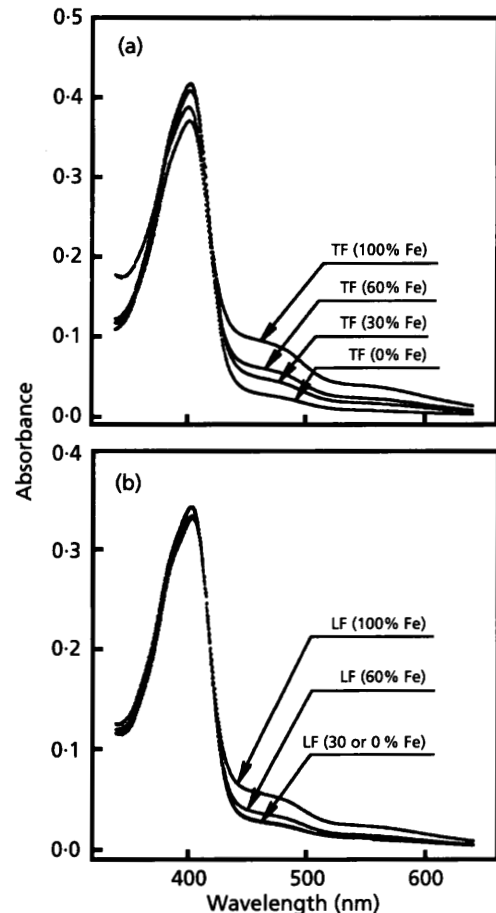


Fig. 5. Absorption spectra (340–640 nm) of *P. aeruginosa* pyoverdin Pa-C and its transferrin (TF) (a) or lactoferrin (LF) reaction mixture (b) at physiological pH. Pyoverdin Pa-C was added to 2.0 ml 100 mM HEPES buffer (pH 7.4) to a final concentration of 20.0 μ M. Transferrin and lactoferrin with various iron saturation levels were added to final concentrations of 20.0 μ M. After incubation at 25 °C for 24 h, the reaction mixtures were ultrafiltered with a micro-concentrator (10000 Da cut-off), and the absorption spectra were measured.

iron-pyoverdin complexes were formed in mixtures containing, 30%, 60% and 100% iron-saturated transferrin.

However, as shown in Fig. 4(b), 30% iron-saturated lactoferrin did not quench pyoverdin fluorescence, while 60% and 100% lactoferrin quenched the fluorescence by approximately 3.9% and 5.8%, respectively. Even when the concentration of pyoverdin and lactoferrin were increased up to 20.0 μ M, pyoverdin was unable to remove iron from 30% iron-saturated lactoferrin. Iron removal was only observed with 60% and 100% iron-saturated lactoferrin, after 24 h incubation at 25 °C (Fig. 5b).

Using protein prepared as described above, the amount of iron bound to transferrin was estimated by absorption spectroscopy (extinction coefficient), ferrozine assay, and the inductively coupled plasma atomic emission

Table 2. Estimation of protein-bound iron using absorption, ferrozine and ICP methods

Transferrin iron saturation (%) (0.1 mM TF)	Iron concn (μM)		
	A_{470} *	Ferrozine	ICP
30	55.9	48.1	48.4
60	111.4	112.6	108.4
100	175.8	185.4	187.6

* $\epsilon_{470} = 0.51$ (1%).

spectroscopic technique (ICP). The results are presented in Table 2. We expected the ferrozine and ICP method values to be significantly higher than the A_{470} values if non-specific binding was a problem in our experimental system. As shown in Table 2 higher values were obtained only at 100% iron saturation in the 5–6% range, considered to lie well within the experimental error. The percentage of iron removed (calculated from Fig. 5a) at 30%, 60% and 100% iron saturation was 24.6%, 19.8% and 24.4%, respectively. At 100% iron saturation the percentage iron removed from transferrin was much higher than the 5–6% observed between the A_{470} and ferrozine methods (Table 2). Taken together the above observations suggested that non-specific binding of iron (if any) probably played no significant role in our experiments. Also, based on these observations, a strong case can be made that non-specific binding should not be a problem with lactoferrin especially since lactoferrin, unlike transferrin, does not readily release its iron (Barclay, 1985; Finkelstein *et al.*, 1983).

DISCUSSION

It should be pointed out that the technique used in this study where iron acquisition from transferrin and lactoferrin was monitored by following pyoverdin fluorescence quenching (Xiao & Kisaalita, 1995), may be advantageous in comparison to the traditional ^{55}Fe membrane dialysis and spectrometric methods in two ways. Firstly, since this is the first report where iron acquisition by pyoverdin has been observed at low protein iron saturation levels (< 100%), the technique may be more sensitive. Secondly, given that low pyoverdin concentration (1.0 μM , similar to levels detected in CF sputum samples) could sufficiently remove Fe^{3+} from transferrin and lactoferrin at physiological pH, it is possible to conduct iron acquisition experiments under *in vivo* conditions.

The results reported in this study conclusively show that *P. aeruginosa* cell growth and pyoverdin production in the presence of iron–transferrin and iron–lactoferrin are affected in a manner corresponding to the level of the iron–protein saturation (Figs 1, 2 and 3). Pyoverdin can acquire iron from transferrin (30–100% iron-saturated) and lactoferrin (60–100% iron-saturated) at physiological pH (Figs 4 and 5). These findings strongly suggest that pyoverdin plays an important role in iron release

from these iron-binding proteins, and are consistent with previous findings that *P. aeruginosa* pyoverdin can acquire iron from 100% iron-saturated transferrin at physiological pH without elastase or pH changes (Wolz *et al.*, 1994; Meyer *et al.*, 1996).

The ability of siderophores to acquire host iron has been related to their capacity to remove iron from transferrin. Also, it has been shown previously by the ^{55}Fe equilibrium technique (Sriyosachati & Cox, 1986) that pyoverdin is not capable of acquiring iron from transferrin at physiological pH, which is at odds with both our results (Figs 4 and 5) and those recently published by Wolz *et al.* (1994) and Meyer *et al.* (1996). This discrepancy may be due to the fact that iron saturation of transferrin was low (only 9.4%, calculated from 12 μg 88% iron-saturated transferrin ml^{-1} , plus 100 μg apo-transferrin ml^{-1}) and the concentration of transferrin was also low (1.4 μM , 112 μg ml^{-1}) (Sriyosachati & Cox, 1986). This may also explain why Döring and co-workers observed iron acquisition in their later work (Wolz *et al.*, 1994) but not in their earlier work (Döring *et al.*, 1988). Transferrin (100% iron-saturated) was used in their later work (Wolz *et al.*, 1994) but only 10% iron-saturated transferrin [0.2 bound iron (mol protein) $^{-1}$] was used in their earlier work (Döring *et al.*, 1988). Taken together, our findings suggest that the concentration of proteins and the degree of iron saturation are important factors in iron acquisition by *P. aeruginosa* pyoverdin.

Lactoferrin is an important iron-binding protein found in most major secretions that bathe mucosal surfaces (Barclay, 1985; Crichton & Charlotiaux-Wauters, 1987; Weinberg, 1978). Lactoferrin has an affinity for iron similar to that of transferrin. However, unlike transferrin, it does not readily release its iron at low pH (Barclay, 1985; Finkelstein *et al.*, 1983). Our data indicated that 60–100% iron-saturated lactoferrin was able to stimulate *P. aeruginosa* growth (Fig. 1) and purified pyoverdin could remove iron from 60% and 100% iron-saturated lactoferrin at physiological pH (Figs 4 and 5). Also, the level of cell growth stimulation and iron release were lower in comparison to those observed with transferrin at similar iron saturation levels. Therefore, the previous results suggesting that pyoverdin does not solubilize iron from lactoferrin even at low pH (Sriyosachati & Cox, 1986) may also have been due to the fact that lactoferrin concentration as well as iron saturation were low. Further work will be required to determine whether pyoverdin can remove iron from transferrin and lactoferrin *in vivo*. The need of the bacterium to sequester ferric iron via pyoverdin may thus result in the development of strategies for better control of *P. aeruginosa* and therefore result in decrease of the corresponding morbidity and mortality seen in CF patients.

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tion of brand names is for information only and does not imply endorsement.

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