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Role of Pyoverdine$_{Pf}$, the Iron-binding Fluorescent Pigment of *Pseudomonas fluorescens*, in Iron Transport

By J. M. MEYER AND J. M. HORNSPERGER

Laboratoire de Biochimie Microbienne, 4 rue Blaise Pascal, Université Louis Pasteur, 67070 Strasbourg Cedex, France

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Uptake experiments with $^{59}Fe^{3+}$ showed that *Pseudomonas fluorescens* had an active system for iron transport. When the purified iron-binding pigment synthesized by this bacterium was added to the external medium, the rate of iron uptake by the cells increased significantly.

INTRODUCTION

Pyoverdine$_{Pf}$, the yellow–green, fluorescent pigment of *Pseudomonas fluorescens*, is synthesized and excreted by iron-deficient cells and has a high specific affinity for Fe^{3+} (Meyer & Abdallah, 1978). Partial determination of its structure has shown that an unusual amino acid, δ-*N*-hydroxyornithine, is present in a cyclic peptide chain (Meyer, 1977). This amino acid is also a constituent of several hydroxamate iron-binding compounds: ferrichrome A (Zalkin *et al.*, 1966); rhodotorulic acid (Atkin & Neilands, 1968); coprogen (Keller-Schierlein & Diekmann, 1970); fusarinine (Sayer & Emery, 1968); ferribactin (Maurer *et al.*, 1968). These siderophores facilitate iron transport into micro-organisms (Neilands, 1974). We show in this paper that iron transport by *P. fluorescens* is an active process, and that it is facilitated by the presence in the external medium of purified pyoverdine$_{Pf}$.

METHODS

Biological material. The producing organism, its growth conditions and the purification of pyoverdine$_{Pf}$ have been described previously (Meyer & Abdallah, 1978).

Experiments on iron uptake. Experiments on $^{59}Fe^{3+}$ uptake were conducted at 25 °C in succinate or citrate media depleted of iron by the technique of Waring & Werkman (1942). Bacteria were harvested in the early exponential phase, centrifuged at 18000 rev. min^{-1} in the cold for 10 min, washed twice by centrifugation with distilled water, and resuspended just before use in similar iron-depleted medium to a concentration of 0·1 mg dry wt ml^{-1}. Some 5 to 10 min after the suspensions had been prepared, $^{59}Fe^{III}$-citrate (20 mCi mg^{-1}) was added to give 5 ng ml^{-1}. (Under these conditions $^{59}Fe^{3+}$ remained in solution throughout the course of the experiments.)

In experiments to study the effect of pyoverdine$_{Pf}$ on iron uptake, increasing quantities of the pure pigment (Meyer & Abdallah, 1978) were mixed with the solutions containing $^{59}Fe^{3+}$ 15 min prior to addition to the cell suspension. This ensured that formation of the Fe(III)–pigment complex was complete before the start of experiments.

Radioassays. Incorporation of $^{59}Fe^{3+}$ by cell suspensions was measured according to Peters & Warren (1968). Samples (1 ml) were periodically withdrawn and very rapidly filtered on membrane filters (0·45 μm porosity). The cells were washed twice with 2 ml of iron-depleted medium, and the filter membranes were then coated with aluminium foil. Radioactivity was measured with a Gammamatic counter (SAIP-CGR, 75015 Paris, France).

Data on iron uptake are expressed as ng $^{59}Fe^{3+}$ incorporated per mg dry wt bacteria. In each experiment, the conversion of radioactivity to weight was based on the determination of radioactivity in a control, consisting of 1 ml of the unfiltered incubation medium containing a known quantity of $^{59}Fe^{3+}$.

Chemicals. Succinic acid and citric acid (analytical reagent grade) were purchased from Merck, and ^{59}Fe (as ferric citrate) from CEA (91190 Gif-sur-Yvette, France).

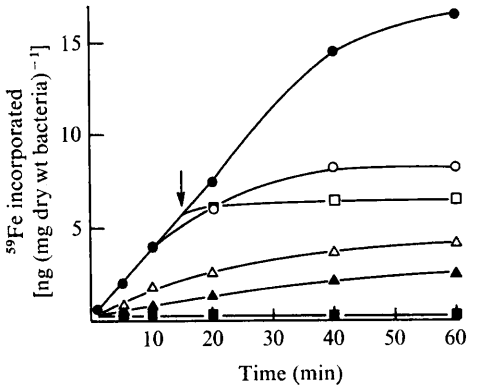

Fig. 1

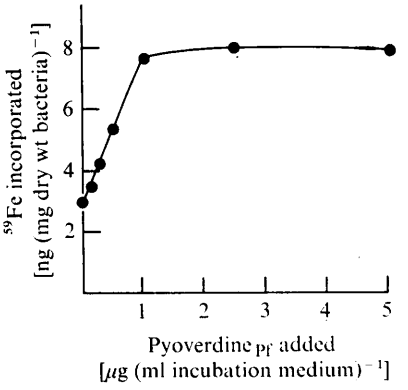

Fig. 2

Fig. 1. Incorporation of ^{59}Fe by *P. fluorescens*: in standard succinate medium at 25 °C (●) or 0 °C (■); in standard medium without succinate (○); in standard succinate medium with added 10 mM-2,4-dinitrophenol (▲), or with added 1 mM-sodium azide plus 1 mM-iodoacetamide (△), or with 0·1 % (v/v) toluene added after 15 min, see arrow (□).

Fig. 2. Effect of pyoverdine$_{Pf}$ on ^{59}Fe incorporation in 60 min by *P. fluorescens*.

RESULTS

Evidence for an active system of iron transport of P. fluorescens

Iron uptake by *P. fluorescens* was markedly stimulated in the presence of an exogenous organic substrate and was strongly temperature dependent: no uptake occurred at 0 °C (Fig. 1). The rate of uptake was reduced 75 to 80% by 2,4-dinitrophenol (10 mM), or sodium azide (1 mM) plus iodoacetamide (1 mM). Addition of 0·1 % (v/v) toluene to a cell suspension rapidly abolished iron uptake (Fig. 1).

Role of pyoverdine$_{Pf}$ in iron transport

As shown previously, growth of *P. fluorescens* in the standard citrate medium without added iron completely repressed fluorescent pigment synthesis (Meyer & Abdallah, 1978). Thus, in experiments to study the influence of the pigment on iron uptake, the bacteria were grown in this medium. The cells were resuspended in iron-depleted citrate medium and supplied with increasing quantities of the purified pigment. The rate of iron uptake by the bacteria increased as a function of pigment concentration up to 1 $\mu g\ ml^{-1}$; under the experimental conditions employed, the rate of iron uptake increased slightly over twofold (Fig. 2). The rate-saturating pigment concentration (1 $\mu g\ ml^{-1}$, 0·66 μM) was, to a good approximation, equimolar to the total amount of Fe^{3+} present in the system [40 ng ml^{-1}, 0·71 μM, = added iron (5 ng ml^{-1}) plus estimated residual iron (35 ng ml^{-1})]. This is in agreement with the 1:1 stoicheiometry of the Fe(III)–pyoverdine$_{Pf}$ complex (Meyer & Abdallah, 1978).

DISCUSSION

Although the ability of certain *Pseudomonas* species to excrete yellow–green, fluorescent, water-soluble pigments is well known, the physiological basis for their production has not hitherto been elucidated. The previous hypotheses of Lenhoff (1963) and Michea-Hamzehpour (1973) that the pigments may be related chemically or functionally to the cytochromes are not borne out by our work, which indicates that the pyoverdine has a specific role in the binding of Fe^{3+} and its transport into *P. fluorescens*.

Together with our previous work (Meyer, 1977; Meyer & Abdallah, 1978), the present paper shows that pyoverdine is a typical microbial iron chelator, i.e. it is a siderophore

(Neilands, 1974). Pyoverdine$_{Pf}$, and siderophores in general, are characterized by the following properties: (i) their synthesis is derepressed only when microbial cells are iron-deficient; (ii) they specifically complex Fe^{3+} and have a weak or negligible affinity for Fe^{2+}; (iii) the Fe(III) complexes have very high stability constants (of the order of 10^{32}); (iv) many siderophores, including pyoverdine$_{Pf}$, contain δ-*N*-hydroxyornithine; (v) as a result of their ability to complex Fe^{3+}, the siderophores increase the rate of entry of this cation into the cell.

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