Role of Pyoverdinepf, the Iron-binding Fluorescent Pigment of Pseudomonas fluorescens, in Iron Transport

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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-Schieflein & 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$^{3+}$-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 GammaMaster 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).
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 μg ml^{-1}; under the experimental conditions employed, the rate of iron uptake increased slightly over twofold (Fig. 2). The rate-saturating pigment concentration (1 μg 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 stoichiometry 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.
Pyoverdine, a desferrisiderophore

(Pyoverdine<sub><i>p</i></sub>, 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<sup>3+</sup> and have a weak or negligible affinity for Fe<sup>2+</sup>; (iii) the Fe(III) complexes have very high stability constants (of the order of 10<sup>39</sup>); (iv) many siderophores, including pyoverdine<sub><i>p</i></sub>, contain δ-N-hydroxyornithine; (v) as a result of their ability to complex Fe<sup>3+</sup>, the siderophores increase the rate of entry of this cation into the cell.

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REFERENCES


