Correlation between Catabolite Repression of Arginine Transport and Repression of Anabolic Ornithine Carbamoyltransferase in *Pseudomonas putida*

By R. P. PIGGOTT AND S. CONDON*

Department of Dairy and Food Microbiology, University College, Cork, Ireland

(Received 26 January 1982; revised 15 March 1982)

Synthesis of anabolic carbamoyltransferase (EC 2.1.3.3.) of *Pseudomonas putida* A90 was repressed two- to five-fold when arginine was added to cultures growing at the expense of a readily utilizable substrate, such as asparagine; synthesis was repressed 16- to 17-fold when arginine was the sole carbon or carbon and nitrogen source. The most likely explanation is that asparagine exerted some form of catabolite repression on arginine transport. Bacteria grown in the presence of asparagine plus arginine transported arginine at only 13% of the rate of bacteria grown at the expense of arginine alone.

INTRODUCTION

The fluorescent pseudomonads are capable of both synthesis and catabolism of arginine. This has obvious regulatory implications. A cell growing in a minimal medium at the expense of some substrate other than arginine might be expected to derepress the biosynthetic pathway, and by allosteric regulation ensure that the intracellular arginine concentration remains insufficient to induce synthesis of enzymes capable of degrading arginine. Addition of arginine to such cells might be expected to reverse this pattern, i.e. to swell intracellular arginine pools, to repress arginine biosynthetic enzymes and possibly (depending on catabolite repression effects between the original growth substrate and the added arginine) to induce arginine catabolism.

However, inclusion of arginine in media in which *Pseudomonas putida* or *Pseudomonas aeruginosa* were growing in the presence of another substrate had only a very slight repressive effect on the enzymes involved in the biosynthesis of arginine. Synthesis of anabolic carbamoyltransferase (aOCTase) was reduced two- to six-fold (Udaka, 1966; Ramos *et al.*, 1967; Isaac & Holloway, 1972; Condon *et al.*, 1976; Legrain *et al.*, 1977; Mercenier *et al.*, 1980), and no other arginine biosynthetic enzyme was repressed (Isaac & Holloway, 1972; Condon *et al.*, 1976). On the other hand, when *P. aeruginosa* was grown on arginine as the sole carbon source a 50- to 100-fold repression of aOCTase was observed (Isaac & Holloway, 1972; Voellmy & Leisinger, 1972; Mercenier *et al.*, 1980). The latter observation was confirmed with *P. putida* in this laboratory, indicating that the repression of synthesis of arginine biosynthetic enzymes in fluorescent pseudomonads can be extensive in certain circumstances.

The work reported here is an examination of possible factors which might explain extensive repression of enzyme synthesis when arginine is the sole growth substrate, but very little repression when an alternative growth substrate is available.

METHODS

Organism. *Pseudomonas putida* strain A90 was originally obtained from the Department of Bacteriology and Immunology, University of California, Berkeley, California, U.S.A. The numbering is that assigned in the classification of Stanier *et al.* (1966).

Media and growth conditions. The minimal medium used contained, per litre: KH₂PO₄, 6.8 g; Na₂HPO₄, 7.1 g; (NH₄)₂SO₄, 1.0 g; MgSO₄ · 7H₂O, 0.6 g; CaCl₂ · 2H₂O, 7.9 mg; FeSO₄ · 7H₂O, 7 mg; nitrilotriacetic acid, 0.2 g. Substrates used as sources of carbon or of carbon and nitrogen [instead of (NH₄)₂SO₄], or as supplements to growing cultures, were added after separate sterilization.
For growth experiments, liquid cultures (150 ml or less) in 500 ml Erlenmeyer flasks were incubated at 30 ± 0.2 °C in a reciprocal shaking water bath at 100 strokes min⁻¹. Growth was monitored by measurement of absorbance at 660 or 580 nm, with a Pye Unicam SP500 spectrophotometer and a Bausch and Lomb Spectronic 20 spectrophotometer, respectively. Specific growth rates, \( k \) (h⁻¹) were calculated as

\[
2.303 \log_{10} \left( \frac{x_2}{x_1} \right) = (t_2 - t_1),
\]

where \( x \) was the absorbance measurement at time \( t \).

**Cell-free extracts.** Extracts were prepared from sonically disrupted cell suspensions (prepared using an MSE sonic disintegrator model 100 W, for two periods of 10 s at maximum intensity, 0 °C) in 10 mM-Tris/HCl buffer, pH 8.5, containing 5 mM-mercaptoethanol. Protein concentrations were determined by the Lowry method, with bovine serum albumin as standard.

**Enzyme assays.** Anabolic ornithine carbamoyltransferase (aOCTase; EC 2.1.3.3) was assayed at pH 9.0, as described previously (Condon et al., 1976). At pH 9.0 a catabolic OCTase also present in this strain had only 5% of its maximal activity (Piggott, 1979). \( N^\alpha \)-Acetylgornithine \( \delta \)-transaminase (EC 2.6.1.11) was assayed according to Albrecht & Vogel (1964). \( N^\alpha \)-acetylgornithine : glutamate transacetylase (EC 2.3.1.35) was assayed according to Udaka & Kinoshita (1958). For each assay a unit of enzyme activity was expressed in μmol of substrate reacting min⁻¹.

**Utilization and transport of arginine.** Utilization of arginine in the presence of other growth substrates in the culture media was measured by assaying for arginine in culture filtrates using the method of Rosenberg et al. (1956), and confirmed by adding [U-\( ^{14} \)C]arginine to the growth medium and assaying (1) the amount of radioactivity taken up by the cells, (2) the amount remaining in the filtrate and (3) the amount respired to CO₂. The amount of isotope added to the culture was 0.02 μCi ml⁻¹ (740 Bq ml⁻¹). Samples of the culture were rapidly harvested by membrane filtration and washed three times with 0.01 M-phosphate buffer pH 7.0 at room temperature. The filter pads containing the washed cells and samples of the filtrate were counted in toluene/Triton X100 scintillation fluid containing 0.25 g POPOP 1⁻¹ and 8.25 g PPO 1⁻¹, using a Beckman LS-7000 scintillation counter. The amount of \( ^{14} \)CO₂ respired from [U-\( ^{14} \)C]arginine was determined in a separate experiment by sparging a culture with sterile air and passing the effluent air through a CO₂-trapping solution, ethanolamine/methanol (30:70, v/v). Samples of the trapping solution were scintillation-counted as above.

The ability of cells to transport arginine was measured by an adaptation of the method of Rahman & Clarke (1980). Washed cells obtained from exponentially growing cultures were suspended in the salts portion of minimal medium (30 °C) containing [U-\( ^{14} \)C]arginine. Samples were removed at 1 min intervals and separated from the labelled arginine medium by rapid membrane filtration and washing. The filter pads were immediately transferred to 10 ml samples of scintillation fluid (as above) and counted in the scintillation counter.

**RESULTS**

When *P. putida*, growing exponentially with asparagine (3 mg ml⁻¹) as the carbon source, was supplemented with arginine (0-1 mg ml⁻¹) the specific activity of aOCTase decreased from 0.94 to 0.44 units (mg protein)⁻¹ in 3 h, i.e. approximately a twofold repression. A similar low degree of repression was noted when cultures growing at the expense of succinate or glucose were supplemented with arginine (0-1 mg ml⁻¹). However, a 17-fold repression of the same enzyme occurred under similar conditions when arginine (3 mg ml⁻¹) was the sole carbon source or carbon and nitrogen source (Table 1).

Several intermediates of the known arginine catabolic pathways in pseudomonads (Miller & Rodwell, 1971; Mercenier et al., 1980; Rahman et al., 1980) were tested as sole carbon sources to determine if one of the intermediates of catabolism, and not arginine itself, was causing the high level of repression. None of the components tested caused as much repression of the aOCTase as arginine itself (Table 1). The maximum level of repression observed with any of the arginine catabolites was about 3-fold, compared to 17-fold repression in cells grown on arginine as the sole carbon source. No repression effects were observed when the activities of two other arginine biosynthetic enzymes, \( N^\alpha \)-acetylgornithine \( \delta \)-transaminase and \( N^\alpha \)-acetylgornithine : glutamate transacetylase were measured in extracts of cells grown on various carbon sources, including arginine and some intermediates of arginine catabolism. The activity of the transacetylase was essentially the same irrespective of the carbon source being utilized. The activity of the transaminase was essentially the same in extracts of cells grown at the expense of asparagine, succinate or glutamate. However, slightly higher activities were noted in cells grown on arginine catabolites and a threefold higher activity obtained in extracts from cells grown on arginine
Role of arginine transport in repression

Fig. 1. Diauxic growth measured as $A_{580}$ (●) and specific activity of aOCTase (○) in *P. putida* A90 growing in a minimal medium containing asparagine (0.5 mg ml$^{-1}$) and arginine (3 mg ml$^{-1}$). The inoculum was an overnight minimal asparagine culture.

Table 1. Specific growth rates of cells and specific activities of aOCTase and $N^\delta$-acetylornithine $\delta$-transaminase of *P. putida* A90 growing at the expense of various substrates, including intermediates of arginine catabolism, as carbon sources

<table>
<thead>
<tr>
<th>Carbon source (3 mg ml$^{-1}$)</th>
<th>Specific growth rate, $k$ (h$^{-1}$)</th>
<th>Specific activities [units (mg protein)$^{-1}$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asparagine</td>
<td>0.93</td>
<td>1.00</td>
</tr>
<tr>
<td>Succinate</td>
<td>0.73</td>
<td>1.08</td>
</tr>
<tr>
<td>Glutamate</td>
<td>0.82</td>
<td>0.8</td>
</tr>
<tr>
<td>Arginine</td>
<td>0.46</td>
<td>0.06*</td>
</tr>
<tr>
<td>Citrulline</td>
<td>0.69</td>
<td>0.46</td>
</tr>
<tr>
<td>Ornithine</td>
<td>0.37</td>
<td>0.48</td>
</tr>
<tr>
<td>Agmatine</td>
<td>0.69</td>
<td>0.6</td>
</tr>
<tr>
<td>Putrescine</td>
<td>0.69</td>
<td>1.92</td>
</tr>
<tr>
<td>Spermidine</td>
<td>0.43</td>
<td>1.00</td>
</tr>
<tr>
<td>Guanidinobutyrate</td>
<td>0.69</td>
<td>0.3</td>
</tr>
<tr>
<td>$\gamma$-Aminobutyrate</td>
<td>0.86</td>
<td>0.9</td>
</tr>
</tbody>
</table>

ND, Not determined.
* A similar value was obtained when arginine was used as the source of both carbon and nitrogen.

(Table 1), indicating induction, rather than repression, by arginine. Induction of this enzyme has already been observed in *P. aeruginosa* (Voellmy & Leisinger, 1978).

The degree of repression caused by arginine supplementation of cultures growing at the expense of an alternative carbon source (e.g. succinate) increased with increasing concentration of arginine. However, the effect was not dramatic; the degree of repression increased from 3-fold to 5-fold for a 30-fold (0.1 to 3.0 mg ml$^{-1}$) increase in arginine supplement concentration.

*Pseudomonas putida* A90 grew twice as fast at the expense of asparagine as it did with arginine (Table 1). The possibility that asparagine utilization might inhibit arginine uptake from the growth medium, and thus affect observed levels of repression was investigated. Growth of *P. putida* was diauxic in a medium containing both asparagine and arginine (Fig. 1). The asparagine was utilized first; a lag period followed and then growth occurred at the expense of arginine. This was confirmed by using [U-$^{14}$C]arginine in the growth medium. Uptake of the label from the culture into cells, or respiration to give $^{14}$CO$_2$ was minimal until the asparagine was utilized; uptake and respiration of the label increased towards the end of the diauxic lag and rapid
Table 2. Rates of arginine uptake by P. putida A90 grown in the presence of various substrates

[U-14C]Arginine (20 µM) was added to cell suspensions and the rates of uptake measured as in Methods. The possible interference of asparagine in the uptake assay was measured by including 20 µM-asparagine in addition to the 20 µM arginine in one set of assays (see footnote).

<table>
<thead>
<tr>
<th>Growth substrate</th>
<th>Rate of arginine uptake [nmol min⁻¹ (mg dry wt)⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine (1 mg ml⁻¹)</td>
<td>8.6</td>
</tr>
<tr>
<td>Arginine (1 mg ml⁻¹)*</td>
<td>8.0</td>
</tr>
<tr>
<td>Asparagine (2 mg ml⁻¹)</td>
<td>1.6</td>
</tr>
<tr>
<td>Asparagine (2 mg ml⁻¹) + arginine (1 mg ml⁻¹)</td>
<td>1.1</td>
</tr>
<tr>
<td>Malonate (2 mg ml⁻¹)</td>
<td>2.7</td>
</tr>
</tbody>
</table>

* Assayed in the presence of 20 µM-asparagine.

The rate of transport of arginine into exponential phase cells grown at the expense of several substrates was assayed (Table 2). Asparagine did not affect the assay itself. Cells grown at the expense of arginine as the sole carbon source transported arginine five- to eight-fold faster than cells grown at the expense of asparagine. Cells grown in a minimal medium containing both asparagine and arginine, and harvested while substantial amounts of asparagine remained in the culture, transported arginine at the low rates characteristic of cells grown on asparagine alone. This means that cells grown on asparagine media, to which arginine was added as a supplement, were capable of transporting arginine at only 13% of the rate of cells grown on arginine as the sole carbon source. Cells grown on malonate (growth rate slightly lower than that on arginine) transported arginine at rates higher than asparagine-grown cells but yet only one-third the rate of arginine-grown cells, indicating the involvement of arginine induction as well as catabolite repression in the regulation of arginine transport.

DISCUSSION

Assessments of repression of amino acid biosynthetic enzymes are often made by measuring the decrease, if any, in the specific activities of the enzymes on addition of small quantities of an amino acid to cultures growing at the expense of a readily utilizable carbon source such as succinate, asparagine or glucose. This method implies that the small quantities of the amino acid in question are readily transported into the cell to swell substantially the existing intracellular pool so that it can exert an effect on the regulation of transcription. If transport of the amino acid is inhibited by the growth conditions, then extensive repression of the amino acid biosynthetic enzymes may not be expressed following amino acid supplementation of cultures. Repression of aOCTase in the arginine biosynthetic pathway of P. putida A90 illustrates this condition. The work reported here indicates that the extent of repression measured depended on whether the culture was actively catabolizing arginine or not. If arginine was the sole source of carbon, or the sole source of carbon and nitrogen, repression was extensive. If arginine was not being actively catabolized, as occurred in the diauxie experiments (Fig. 1), repression of aOCTase observed was minimal. The diauxie experiments clearly showed that asparagine, which is a readily utilizable growth substrate for P. putida A90, exerted a catabolite repression effect on arginine utilization (Fig. 1). Arginine added as a supplement to cultures growing on asparagine was not readily catabolized. This finding, and the observation that the concentration of arginine could be increased 30-fold with only a minimal effect on the degree of repression, eliminates the possi-
bility that the low level of repression observed when arginine is added as a supplement is due to catabolism of arginine, resulting in its rapid depletion from the growth medium.

The fact that the rate of transport of arginine is subject to catabolite repression (Table 2) explains how cells growing at the expense of arginine could have extensively repressed levels of aOCTase. Such cells could maintain high intracellular concentrations of arginine, or arginine catabolites, whereas cells growing on a readily utilizable substrate (such as asparagine) would have difficulty in maintaining high intracellular arginine pools because of the catabolite repression effect on arginine transport and allosteric regulation of arginine biosynthesis (Udaka, 1966; Isaac & Holloway, 1972; Leisinger et al., 1972).

These findings are supported by the work of Voellmy & Leisinger (1978), who showed that the specific activities of aOCTase and Nε-acetylornithine δ-transaminase were inversely related in P. aeruginosa grown at the expense of different carbon substrates. Lysine competed with arginine for a common transport system in this bacterium. Cultures grown on mixtures of arginine and lysine had levels of aOCTase proportional to the lysine concentration.

_Pseudomonas putida_ grown on malonate (specific growth rate slightly less than that on arginine) transported arginine at only one-third of the rate of arginine-grown cells. This indicates that catabolite repression is not the sole element involved in the regulation of arginine transport. Induction by arginine is also apparently involved, confirming the observation of Fan et al. (1972) with another strain of _P. putida._

Whether the apparent lack of repression of enzymes of biosynthetic pathways other than arginine biosynthesis is due to catabolite repression of amino acid uptake is a question that awaits the outcome of further experiments. Kay & Gronlund (1969a) reported that _P. aeruginosa_ grown on glucose had active transport systems for 18 of the common amino acids. Whether greater transport activities would be observed on cultivation at the expense of each individual amino acid was not determined, except in the case of proline, the transport of which was inducible by proline and subject to partial catabolite repression by glucose (Kay & Gronlund, 1969b).

**REFERENCES**


