A Partial Order for Genes Determining Enzymes of the meta-Cleavage Pathway in *Pseudomonas putida*

By G. J. WIGMORE* and R. C. BAYLY

Department of Microbiology, Monash University Medical School, Commercial Road, Prahran, 3181, Victoria, Australia

(Received 4 October 1976; revised 2 December 1976)

SUMMARY

A partial order for genes which specify meta-cleavage pathway enzymes has been derived from properties of a polarity mutant strain (PsU5) of *Pseudomonas putida* NCIB10015 and a partial revertant, PsU5/R21. The polar mutation is within the 2-hydroxymuconic semialdehyde dehydrogenase gene and results in loss of detectable 2-hydroxymuconic semialdehyde hydrolase and 90 to 95% reduction in the activities of 4-oxalocrotonate tautomerase, 4-oxalocrotonate decarboxylase, 2-oxopent-4-enoate hydratase and 4-hydroxy-2-oxovalerate aldolase. The partial revertant PsU5/R21 regained wild-type levels of all enzymes except the aldehyde dehydrogenase.

It is probable that all genes determining the dehydrogenase and subsequent enzymes are transcribed as a polycistronic message. This is the first report of mapping genes determining the enzymes of the meta-cleavage pathway.

INTRODUCTION

Transduction, conjugation and transformation have been demonstrated in various species of fluorescent pseudomonads (Holloway, 1975; Stanisich & Richmond, 1975) but a system of recombination has not been established for *Pseudomonas putida* NCIB10015 (strain U) (Wigmore, 1975). Gene order may also be elucidated and confirmed by deletion mapping (Wheelis & Ornston, 1972) and from studies of polarity mutants (Ornston, 1966).

*Pseudomonas putida* NCIB10015 grows at the expense of phenol and the isomers of cresol following meta-cleavage of catechol and the methylcatechols respectively. A pleiotropic mutation within the genes determining the enzymes of the meta-cleavage pathway has been reported which is consistent with a polarity mutation (Bayly & Wigmore, 1973). The present work describes how this mutant, PsU5, carrying the pleiotropic mutation, was used to investigate an order for genes determining enzymes of the meta-cleavage pathway.

METHODS

Some properties of wild-type *Pseudomonas putida* NCIB10015, designated PsU0, the pleiotropic mutant PsU5 and the partial revertant PsU5/R21 have been reported previously (Bayly & Wigmore, 1973). Organisms were grown and extracts were prepared as described by Bayly & Wigmore (1973). All enzymic activities were determined as reported by Wigmore & Bayly (1974) except 4-hydroxy-2-oxovalerate aldolase which was determined as described by Feist & Hegeman (1969).

* Present address: Department of Biochemistry, School of Medicine, University of Miami, Florida, U.S.A.
Table 1. Activities of enzymes of the meta-cleavage pathway in extracts of phenol-induced strains

Extracts were assayed for enzymic activities 2 h after phenol (2.5 mM) was added to organisms growing in basal medium containing fumarate (10 mM). No activities were detected without phenol induction. Specific activities (except for tautomerase and decarboxylase) are expressed as nmol substrate used or product formed min⁻¹ (mg protein)⁻¹. Tautomerase activity is expressed as units of activity, where 1 unit represents the decrease of one absorbance unit at 295 nm min⁻¹ (mg protein)⁻¹. Decarboxylase activity is expressed as: +++, activity induced in the wild-type strain; ++, 5 to 10% of the activity induced in the wild-type strain; ±, 0.1 to 1.1 units.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>PsU0</th>
<th>PsU5</th>
<th>PsU5/R21</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3-Oxygenase</td>
<td>140</td>
<td>70</td>
<td>130</td>
</tr>
<tr>
<td>Dehydrogenase</td>
<td>19</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Hydrolase</td>
<td>53</td>
<td>ND</td>
<td>53</td>
</tr>
<tr>
<td>Tautomerase</td>
<td>1</td>
<td>0.1</td>
<td>1.1</td>
</tr>
<tr>
<td>Decarboxylase</td>
<td>+</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>Hydratase</td>
<td>730</td>
<td>52</td>
<td>690</td>
</tr>
<tr>
<td>Aldolase</td>
<td>8</td>
<td>T</td>
<td>7</td>
</tr>
</tbody>
</table>

ND, Not detected.

RESULTS AND DISCUSSION

The mutational defect in mutant PsU5 resulted in either the complete loss or a considerable reduction in the activity of the six enzymes of the meta-cleavage pathway acting later in the degradative sequence than catechol 2,3-oxygenase (Table 1). The frequency of appearance of complete revertants (Bayly & Wigmore, 1973) and the residual activity of several enzymes in mutant PsU5 are inconsistent with either multiple mutations or a deletion mutation. The phenotype of mutant PsU5 is unlikely to be due to a regulatory defect as it is not possible to propose a simple model to account for the partial revertant, in which only the dehydrogenase is inactive. It is also difficult to reconcile a regulatory mutation with partial loss of some enzymic activities and complete loss of others.

The most probable explanation of the pleiotropy of mutant PsU5 is that the phenotype is a consequence of premature termination of either transcription or translation. The residual activities in mutant PsU5 would result from a low efficiency reinitiation of either transcription or translation respectively, and the partial revertants would be the consequence of the spontaneous creation of an internal reinitiation site.

On the basis of the explanation of polarity mutations proposed by Morse & Guertin (1971), termination of mRNA transcription is a less likely explanation of the behaviour of mutant PsU5 than premature termination of translation.

A system analogous to PsU5 has been reported in the his operon of Salmonella typhimurium. A frame-shift mutation located in the hisD gene reduced expression of the adjacent gene, hisC, by 98 to 99% but reduced the expression of subsequent genes by only 90% (Voll, 1967; Rechler & Martin, 1970; Rechler et al., 1972). Reznikoff et al. (1974) proposed a model which explained these observations and also their observations of disproportionate production of β-galactosidase and thiogalactoside transacetylase in trp-lac fusion strains of Escherichia coli. The major premise of this model is that efficient transcription of an operon requires a minimum number of ribosome binding events to ensure propagation and/or protection of the mRNA.

The enzymic activities of mutant PsU5 are consistent with a polarity mutation within the genes determining either the dehydrogenase or hydrolase. To account for the phenotype of...
Fig. 1. A partial gene-order for genes determining enzymes for the catabolism of phenol in *P. putida*. Key to genes coding for enzymes: A, hydroxylase; B, 2,3-oxygenase; C, dehydrogenase; D, hydrolase; E, F, G and H, decarboxylase, tautomerase, hydratase, aldolase (order unknown).
the partial revertants it is necessary to assume that the polar mutation is within the dehydro-
genase gene and that the hydrolase is operator-distal. The partial revertants are explained
by the creation of a new site for initiation of protein synthesis. Translational restarts within
the lacI gene transcript of the lac operon can be created by the introduction of a nonsense
codon before the initiation codon. Initiation occurred at both AUG (Platt et al., 1972) and
non-AUG codons (Ganem et al., 1973; Files, Weber & Miller, 1974).

Any explanation of the phenotype of mutant PsU5 and its partial revertant requires that
the genes that determine the dehydrogenase and all subsequent enzymes are transcribed as
a single polycistronic message. These explanations do not directly implicate the catechol
2,3-oxygenase gene transcript as part of this polycistronic message. However, there is no
reason to exclude it, assuming this gene is at the operator-proximal end of the message.

The partial gene-order for genes coding for enzymes of the meta-cleavage pathway
(Fig. 1) is consistent with the properties of mutant PsU5 and its partial revertant PsU5/R21.

The proposed partial gene-order can be tested and the gene-order further elucidated by
deletion mapping.

The project was supported in part by grant no. D67/16545 from the Australian Research
Grants Commission. We thank J. May and M. Barbour for helpful discussions.

REFERENCES

Bayly, R. C. & Wigmore, G. J. (1973). Metabolism of phenol and cresols by mutants of Pseudomonas

pathway of Pseudomonas putida: the regulon is composed of two operons. Journal of General Micro-
biology 100, 71–79.


fragments at three internal sites early in the lac i gene of Escherichia coli. Proceedings of the National Academy

at a codon other than AUG. Proceedings of the National Academy of Sciences of the United States
of America 70, 3165–3169.

Holloway, B. W. (1975). Genetic organization of Pseudomonas. In Genetics and Biochemistry of Pseudo-


Ornston, L. N. (1966). The conversion of catechol and protocatechuate to β-ketoadipate by Pseudomonas

repressor fragment. Proceedings of the National Academy of Sciences of the United States of America 69,
897–901.


