REGULATION OF THE META-CLEAVAGE OF 4-HYDROXYPHENYLACETIC ACID BY PSEUDOMONAS PUTIDA

M.G. BARBOUR AND R.C. BAYLY

DEPARTMENT OF MICROBIOLOGY

MONASH UNIVERSITY MEDICAL SCHOOL,

COMMERCIAL ROAD, PRAHRAN, VICTORIA, 3181, AUSTRALIA

Received April 4,1977

SUMMARY

Pseudomonas putida wild-type strain P23X1 degrades 4-hydroxyphenylacetic acid by an inducible meta-cleavage pathway. Induction experiments with three mutant strains blocked at different reactions in the pathway indicate that the enzymes are synthesized following two sequential inductive events. 4-Hydroxyphenylacetic acid induces the 4-hydroxyphenylacetic acid-3-hydroxylase. 3,4-Dihydroxyphenylacetic acid induces at least the three subsequent enzymes (3,4-dihydroxyphenylacetic acid 2,3-dioxygenase, 5-carboxymethyl-2-hydroxymuconic semialdehyde dehydrogenase, and 5-carboxymethyl-2-hydroxymuconic acid decarboxylase). Thus, the genes encoding the first four enzymes of this meta-cleavage pathway appear to exist as two operons.

INTRODUCTION

The meta-cleavage pathway (Fig. 1) of 4-hydroxyphenylacetic acid in two strains of Pseudomonas putida and an Acinetobacter strain has been elucidated by Sparnins et al (1). This meta-cleavage pathway closely resembles the meta-cleavage pathway for p-cresol degradation with respect both to the types and sequence of reactions (2). There is no evidence of a comparable tautomerase-mediated reaction in the 4-hydroxyphenylacetic acid meta-cleavage pathway (1). Studies of the regulation of meta-cleavage degradation of aromatic compounds have been confined to the meta-cleavage of phenol and the isomers of cresol (3,4,5,6,7). In this communication we report studies on the regulation of synthesis of some enzymes involved in the meta-cleavage pathway of 4-hydroxyphenylacetic acid by P. putida strain P23X1 employing mutant

ISSN 0006-291X

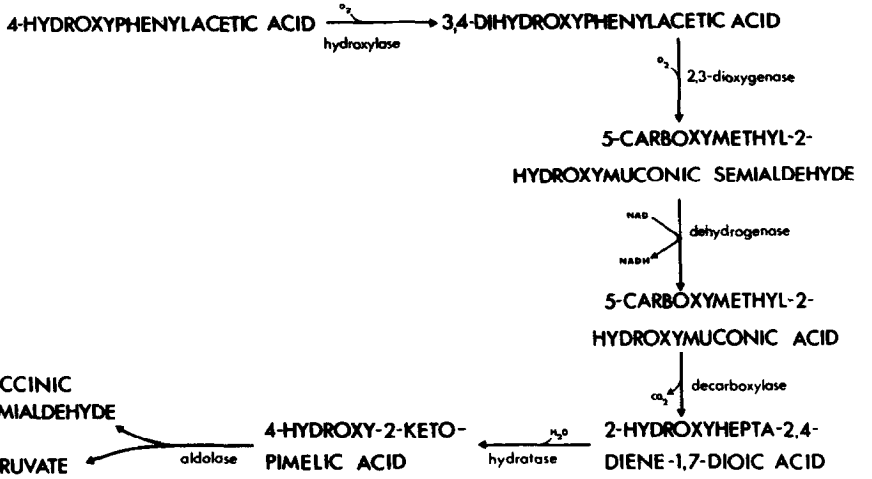

Figure 1. The meta-cleavage pathway for the degradation of 4-hydroxyphenylacetic acid.

strains defective in different reactions of the pathway.

MATERIALS AND METHODS

Organisms and conditions of growth. The mutant strains used are listed in Table 1 and were all derived from wild-type P. putida strain P23X1 (NCIB 9865). This organism was isolated on 2,3-xylenol by Dr. D. Hopper, who has reported some of its metabolic activities (8). Cultures were grown in the basal medium of Hegeman (9) supplemented with 10 mM fumarate. Inducing carbon sources were added to cultures growing on fumarate basal medium to a final concentration of 2.5 mM. Conditions of growth, induction of enzymes and preparation of cell suspensions were as described by Bayly and Wigmore (2) except that induction was for 4 hr. Extracts of freshly harvested cells were prepared by sonic disintegration at 0-2° using a Branson B12 sonifier at an output of 20 KHz for three 10 sec. periods with 1 min. intervals.

Mutagenesis and selection of mutant strains. Exponential phase nutrient broth cultures were exposed to 2%(v/v) ethylmethane

Table 1. Mutant strains used.

Strain	Phenotype
P23X6	hydroxylase deficient.
P23X28	2,3-dioxygenase deficient.
P23X21	dehydrogenase deficient.

sulphonate for 30 min. at 30° which resulted in a 95 to 99% reduction in the number of viable cells. Following overnight expression in nutrient broth, mutant strains unable to grow at the expense of 4-hydroxyphenylacetic acid were selected by the penicillin G (5×10^4 U/ml)/D-cycloserine (2 mg/ml) method of Ornston et al (10). The selective carbon source was quinic acid (2.5 mM) and the contra-selective carbon source was 4-hydroxyphenylacetic acid (2.5 mM). The three mutant strains used in this study were derived by this method.

Enzyme assays. 2,3-dioxygenase and dehydrogenase activities were determined by the method of Sparnins et al (1). Determinations of decarboxylase and hydratase activities were based on the methods of Sala-Trepat and Evans (11) and Collinsworth et al (12) respectively. The preparation of substrates for the assays of dehydrogenase, decarboxylase and hydratase have been described (1). Hydroxylase activity in cell suspensions was determined polarographically using a Clark oxygen electrode as described by Bayly and McKenzie (13).

RESULTS

P. putida strain P23X1 metabolizes 4-hydroxyphenylacetic acid by the meta-cleavage pathway elucidated by Sparnins et al (1) to succinic semialdehyde, pyruvate and CO_2 (Barbour, unpublished observations). All enzymes tested that are involved in the degra-

Table 2. Specific activities of 4-hydroxyphenylacetic acid meta-cleavage pathway enzymes induced by 2.5 mM 4-hydroxyphenylacetic acid and 2.5 mM 3,4-dihydroxyphenylacetic acid in wild-type strain P23X1 and mutant strain P23X6.

	P23X1		P23X6	
	4-HPA[a]	3,4-DHPA[a]	4-HPA[a]	3,4-DHPA[a]
hydroxylase[b]	118.4	<5	<5	<5
2,3-dioxygenase[c]	0.84	0.463	<0.001	0.241
dehydrogenase[c]	0.078	0.056	<0.001	0.048
decarboxylase[c]	0.016	0.016	<0.001	0.013
hydratase[c,d]	0.040	0.021	<0.001	0.015

a. Abbreviations: 4-HPA, 4-hydroxyphenylacetic acid; 3,4-DHPA, 3,4-dihydroxyphenylacetic acid.
b. Values, corrected for endogenous respiration, are expressed as microlitres of oxygen taken up per hour per mg. dry weight of cells.
c. Expressed as micromoles of substrate used (or product formed) per minute per mg. protein.
d. Corrected for non-enzymatic breakdown of substrate.

dation of 4-hydroxyphenylacetic acid were induced when fumarate-grown wild-type strain P23X1 was exposed to 2.5 mM 4-hydroxyphenylacetic acid (Table 2). When a fumarate-grown culture of mutant strain P23X6 was exposed to 2.5 mM 4-hydroxyphenylacetic acid none of the meta-pathway enzymes tested were synthesized. Exposure of fumarate-grown cultures of both these strains to 2.5 mM 3,4-dihydroxyphenylacetic acid resulted in all enzymes tested except the hydroxylase being induced, (Table 2). These results indicate that mutant strain P23X6 does not synthesize a functional hydroxylase in response to 4-hydroxyphenylacetic acid, and the failure of this compound to induce any enzymes in strain P23X6 (Table 2) suggests that the inductive function of 4-hydroxyphenylacetic acid is restricted to the hydroxylase.

Exposure of fumarate-grown cultures of mutant strains P23X28 and P23X21 to 2.5 mM 3,4-dihydroxyphenylacetic acid resulted in

Table 3. Specific activities of 4-hydroxyphenylacetic acid meta-cleavage pathway enzymes induced by 2.5 mM 3,4-dihydroxyphenylacetic acid in wild-type strain P23X1, and mutant strains P23X28 and P23X21.

	Strain		
	P23X1	P23X28	P23X21
2,3-dioxygenase[a]	0.535	<0.001	0.272
dehydrogenase[a]	0.062	0.034	<0.001
decarboxylase[a]	0.010	0.007	0.008
hydratase[a,b]	0.017	0.012	0.013

a. Expressed as micromoles of substrate used (or product formed) per minute per mg. protein.
b. Corrected for non-enzymatic breakdown of substrate.

all enzymes tested except the 2,3-dioxygenase and dehydrogenase, respectively, being synthesized (Table 3). These results suggest that at least the 2,3-dioxygenase, dehydrogenase and decarboxylase are subject to coincident control with synthesis elicited by 3,4-dihydroxyphenylacetic acid.

DISCUSSION

The first enzyme in the pathway, the hydroxylase, is induced by its substrate, 4-hydroxyphenylacetic acid, while the three subsequent enzymes are coincidentally regulated with synthesis elicited by the second metabolite, 3,4-dihydroxyphenylacetic acid. 3,4-Dihydroxyphenylacetic acid does not have any inductive effect on the expression of the hydroxylase gene and it is not yet known whether this compound or a subsequent metabolite induces the synthesis of the hydratase or aldolase enzymes. These results indicate that the genes encoding the first four enzymes of the 4-hydroxyphenylacetic acid meta-pathway exist as two operons, one containing the hydroxylase gene while the other contains at least the 2,3-dioxygenase, dehydrogenase and decarboxylase genes.

This mode of regulation by two sequential inductive events differs from the regulation of the meta-cleavage pathway for phenol degradation in P. putida strain U (3,4,5,13), P. aeruginosa strain T1 (14) and P. arvilla mt-2 (15) where the primary metabolite, phenol, elicits the coincidentally regulated synthesis of all enzymes in the pathway. By the definition of Maas and Clark (16) the genes encoding the phenol meta-cleavage enzymes exist as a single regulon with synthesis elicited by phenol. Bayly et al (6) showed that in P. putida strain U this regulon is composed of two operons, the first contains the phenol hydroxylase gene and the second contains the genes for all subsequent enzymes of the meta-pathway. Phenol is the effector compound for both operons and it has been suggested (3,7) that the expression of both operons is mediated by the same regulatory molecule. In P. putida strain P23X1 at least the first four enzymes in the 4-hydroxyphenylacetic acid meta-cleavage pathway appear to exist as two operons with each operon having different effector compounds. It is not known whether the expression of these two operons is mediated by different regulatory molecules.

ACKNOWLEDGEMENTS

The work was supported in part by an A.R.G.C. Grant No. D67/16545.

REFERENCES

1. Sparnins, V.L., Chapman, P.J. and Dagley, S. (1974) J. Bacteriol. 120, 159-167.
2. Bayly, R.C. and Wigmore, G.J. (1973) J. Bacteriol. 113, 1112-1120.
3. Wigmore, G.J. and Bayly, R.C. (1974) Biochem. Biophys. Res. Commun. 60, 48-55.
4. Feist, C.F. and Hegeman, G.D. (1969) J. Bacteriol. 100, 869-877.
5. Sala-Trepat, J.M., Murray, K. and Williams, P.A. (1972) Eur. J. Biochem. 28, 347-356.
6. Bayly, R.C., Wigmore, G.J. and McKenzie, D.I. (1977) J. Gen. Microbiol. 100,
7. Wigmore, G.J., Di Berardino, D. and Bayly, R.C. (1977) J. Gen. Microbiol. 100,

8. Hopper, D.J. (1967) Ph.D. Thesis. University of Hull.
9. Hegeman, G.D. (1966) J. Bacteriol. 91, 1140-1154.
10. Ornston, L.N., Ornston, M.K. and Chou, G. (1969) Biochem. Biophys. Res. Commun. 36, 179-184.
11. Sala-Trepat, J.M. and Evans, W.C. (1971) Eur. J. Biochem. 20, 400-413.
12. Collinsworth, W.L., Chapman, P.J. and Dagley, S. (1973) J. Bacteriol. 113, 922-931.
13. Bayly, R.C. and McKenzie, D.I. (1976) J. Bacteriol. 127, 1098-1107.
14. Ribbons, D.W. (1970) Arch. Mikrobiol. 74, 103-115.
15. Feist, C.F. and Hegeman, G.D. (1969) J. Bacteriol. 100, 1121-1123.
16. Maas, W.K. and Clark, A.J. (1964) J. Mol. Biol. 8, 365-370.