JOURNAL OF BACTERIOLOGY, Jan. 1987, p. 414–415
0021-9193/87/010414-02$02.00/0

Vol. 169, No. 1

# Benzoate and Muconate, Structurally Dissimilar Metabolites, Induce Expression of *catA* in *Acinetobacter calcoaceticus*

ELLEN L. NEIDLE AND L. NICHOLAS ORNSTON*

*Department of Biology, Yale University, New Haven, Connecticut 06511*

Received 11 August 1986/Accepted 11 October 1986

**Biosynthetic regulation of *catA*, the gene encoding catechol 1,2-dioxygenase (EC 1.13.1.1), was studied in an *Acinetobacter calcoaceticus* mutant strain unable to metabolize benzoate. Benzoate and muconate independently induced the enzyme. In glucose-grown cells, benzoate yielded higher enzyme levels than did muconate, whereas muconate was the more effective inducer in succinate-grown cells.**

Studies of the regulatory effects of metabolites in the β-ketoadipate pathway for catechol dissimilation (Fig. 1) indicate that muconate elicits expression of the *cat* genes in *Acinetobacter calcoaceticus* (2). The action of catechol 1,2-dioxygenase (EC 1.13.1.1), the product of the *catA* gene, on its substrate, catechol, produces muconate, which then triggers increased expression of the gene. Muconate also elicits exression of the *catBCDE* gene cluster, although these genes are not expressed coordinately with the *catA* gene (2, 9). Whether or not benzoate and 2-hydro-1,2-dihydroxybenzoate, the metabolic precursors of catechol (Fig.1), also act as inducers of the *cat* genes in *Acinetobacter* has not been established previously. The availability of mutant strains in which metabolism of benzoate or catechol is completely blocked now permits us to explore this possibility.

In this report we describe the use of a *catA* mutant strain (ADP134) and a mutant deficient in benzoate metabolism (ADP137) to demonstrate that, in addition to muconate, benzoate induces expression of *catA* and, to a lesser extent, *catB* in *A. calcoaceticus*. The mutant strains were selected from the wild-type strain ADP1 (5) as colonies that were resistant to 5 mM 4-fluorobenzoate in the presence of 10 mM succinate. As has been shown, selection for fluorobenzoate resistance can yield spontaneous mutants that are blocked in the catabolism of benzoate (10). Reversion rates of less than 1 in $10^9$ were observed in the two mutant strains.

When strain ADP134 was grown in the presence of benzoate, no catechol 1,2-dioxygenase activity could be detected, and catechol accumulated in the growth medium. Other *cat* genes were not affected by the genetic lesion in ADP134 as indicated by its ability to grow as well as wild-type cells at the expense of muconate and by its expression under this condition of the *catBCDE* genes at wild-type levels. Strain ADP134, which had been selected for its inability to metabolize benzoate, also failed to express *catA* in response to muconate. In addition, recombinants of ADP134 in which the wild-type *catA* gene was acquired by transformation and selected with benzoate as the growth substrate (5) invariably formed catechol 1,2-dioxygenase inducibly in response to muconate. Therefore, a single *catA* gene appears to be expressed in reponse to both metabolites. However, since benzoate is converted to muconate by both the transformants and wild-type cells, the true inducer cannot be determined when these cells are grown in the presence of benzoate.

Strain ADP137 does not metabolize benzoate. As detected by monitoring metabolites in the culture medium by high-performance liquid chromatography, no more than 2% of 1 mM benzoate was removed by $10^9$ mutant cells per ml during

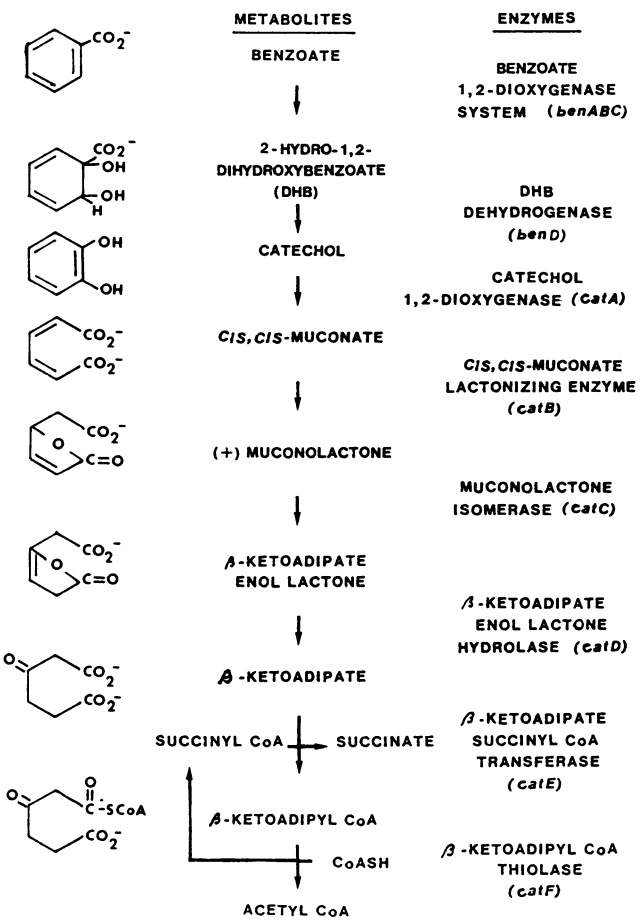


FIG. 1. Metabolites, enzymes, and structural genes associated with dissimilation of benzoate via catechol and the β-ketoadipate pathway. All of the *cat* genes are expressed in response to muconate in *A. calcoaceticus*.

* Corresponding author.

TABLE 1. Expression of *catA* and *catB* in response to benzoate and muconate

| Strain | Ability to metabolize benzoate | Growth substrate | Source of inducer | Relative expression of: | |
|---|---|---|---|---|---|
| | | | | *catA*[a] | *catB*[b] |
| ADP1 | + | Glucose | Benzoate | 100[c] | 100[c] |
| ADP137 | − | Glucose | Benzoate | 97 | 8 |
| ADP137 | − | Succinate | Benzoate | 18 | 1.5 |
| ADP137 | − | Glucose | Muconate | 12[d] | 85[d] |
| ADP137 | − | Succinate | Muconate | 70[d] | 80[d] |

[a] The specific activity of the *catA* gene product, catechol 1,2-dioxygenase, in wild-type cells after growth with glucose in the presence of benzoate was 0.33 μmol/min per mg of protein. The uninduced level of *catA* expression in wild-type cells after growth with succinate (the specific activity of catechol 1,2-dioxygenase) was <0.002 μmol/min per mg of protein.

[b] The specific activity of the *catB* gene product, *cis,cis*-muconate lactonizing enzyme, in wild-type cells after growth with glucose in the presence of benzoate was 0.20 μmol/min per mg of protein. The uninduced level of *catB* expression in wild-type cells after growth with succinate (the specific activity of *cis,cis*-muconate lactonizing enzyme) was <0.002 μmol/min per mg of protein.

[c] Similar levels were observed with succinate as the growth substrate.

[d] Similar levels were observed with wild-type cells.

incubation at 37°C for 2 days. Like its wild-type parental strain, the *ben*-deficient strain ADP137 expressed *catA* at fully induced levels (catechol 1,2-dioxygenase specific activities of between 0.6 and 0.75 μmol/min per mg of protein in crude extracts) during growth with muconate. This mutant, which is fully inducible by muconate but cannot form muconate from benzoate, was then used to determine the ability of benzoate itself to induce expression of *catA*.

The expression, in ADP137, of *catA* and *catB* in response to benzoate and muconate with both glucose and succinate as carbon sources was determined by measuring the respective gene products, catechol 1,2-dioxygenase (3) and *cis,cis*-muconate lactonizing enzyme (6; Table 1). The mutation that prevents benzoate metabolism in ADP137 did not alter the level of expression of the *catA* gene in comparison with that of the wild type when both strains were grown in minimal medium (8) with glucose (10 mM) and benzoate (2 mM). Benzoate analogs that are not metabolized by *A. calcoaceticus* (3-chlorobenzoate and 3-toluate) were about 20% as effective as benzoate in eliciting *catA* expression in either the mutant or the wild-type strain (data not shown). It is likely, therefore, that benzoate itself, rather than a trace of a subsequent metabolite, acts as the inducer.

The pattern of repression, however, was found to be markedly different when muconate and benzoate were compared as inducers in ADP137. With benzoate induction, succinate reduced expression of *catA* by 80% compared with that found in glucose-grown cells. With muconate induction, the effects of glucose and succinate were reversed (Table 1).

Benzoate also had a slight stimulatory effect on *catB* expression in ADP137 (Table 1). The level of *catB* expressed in the presence of benzoate with glucose as the carbon source was 10% of that found in cells induced by muconate. With succinate as the carbon source, benzoate induction produced 2% of muconate-stimulated *catB* expression. Thus, the relative effectiveness of succinate and glucose in repressing *catB* expression was similar to that found for *catA* expression when benzoate was used as an inducer. However, whereas glucose had a strong inhibitory effect on *catA* expression induced by muconate, this effect was not observed when *catB* expression was measured. In this case, activity was the same whether the cells were grown with succinate or glucose (Table 1). All of the effects observed with benzoate as an inducer were also found with the nonmetabolizable benzoate analogs, although the enzyme levels found in each case were about 20% of those produced by benzoate.

These results demonstrated that in *A. calcoaceticus* a single *catA* gene is expressed independently in response to either benzoate or muconate. Induction of the *catA* gene by muconate has been reported previously in fluorescent *Pseudomonas* species (7), as well as in *A. calcoaceticus* (2). In *Pseudomonas putida* NCIB 10015, induction of catechol 1,2-dioxygenase by catechol or muconate and also by benzoate was reported (1), although in these experiments the possible presence of two differently regulated genes was not excluded. Induction of *catA* by benzoate has been reported in *Alcaligenes eutrophus*, a bacterium in which muconate causes expression of *catB* but not *catA* (4).

The observation that two structurally dissimilar compounds can control expression of a single gene suggests that more than one regulatory site is present. The complexity of transcriptional control is further indicated by the differences in repression that were observed when different inducers and carbon sources were used.

This work was supported by the Celanese Research Company, by National Science Foundation grant DMB 8414961, and by Public Health Service grants GM 07223 and GM 33377 from the National Institutes of Health.

We thank M. E. Rae and R. Lerud for isolating mutant strains and R. E. Parales for maintenance of strains.

## LITERATURE CITED

1. **Bayly, R. C., and D. I. McKenzie.** 1976. Catechol oxygenases of *Pseudomonase putida* mutant strains. J. Bacteriol. **127**:1098–1107.
2. **Canovas, J. L., and R. Y. Stanier.** 1967. Regulation of the enzymes of the β-ketoadipate pathway in *Moraxella calcoaceticus*. Eur. J. Biochem. **1**:289–300.
3. **Hayaishi, O., M. Katagiri, and S. Rothberg.** 1957. Studies on oxygenases. Pyrocatechase. J. Biol. Chem. **229**:905–920.
4. **Johnson, B. F., and R. Y. Stanier.** 1971. Regulation of the β-ketoadipate pathway in *Alcaligenes eutrophus*. J. Bacteriol. **107**:476–485.
5. **Neidle, E. L., and L. N. Ornston.** 1986. Cloning and expression of the *Acinetobacter calcoaceticus* catechol 1,2-dioxygenase structural gene *catA* in *Escherichia coli*. J. Bacteriol. **168**: 815–820.
6. **Ornston, L. N,** 1966. The conversion of catechol and protocatechuate to β-ketoadipate by *Pseudomonas putida*. III. Enzymes of the catechol pathway. J. Biol. Chem. **241**:3795–3799.
7. **Ornston, L. N.** 1971. Regulation of catabolic pathways in *Pseudomonas*. Bacteriol. Rev. **35**:87–116.
8. **Ornston, L. N., and R. Y. Stanier.** 1966. The conversion of catechol and protocatechuate to β-ketoadipate by *Pseudomonas putida*. I. Biochemistry. J. Biol. Chem. **241**:3776–3786.
9. **Shanley, M. S., E. L. Neidle, R. E. Parales, and L. N. Ornston.** 1986. Cloning and expression of *Acinetobacter calcoaceticus catBCDE* genes in *Pseudomonas putida* and *Escherichia coli*. J. Bacteriol. **165**:557–563.
10. **Wigmore, G. J., and D. W. Ribbons.** 1980. *p*-Cymene pathway in *Pseudomonas putida*: selective enrichment of defective mutants by using halogenated substrate analogs. J. Bacteriol. **143**:816–824.