Specificity of a Catabolic Pathway—a Lesson Learned from Indirect Assays

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Studies with purified orcinol hydroxylase suggest that, contrary to previous conclusions, the enzymes of the orcinol pathway cannot transform analogous compounds to common metabolites. The substrate analogues of orcinol uncouple electron flow from reduced nicotinamide adenine dinucleotide to oxygen from the hydroxylation reaction catalyzed by orcinol hydroxylase.

We have shown that orcinol is catabolized by Pseudomonas putida 01 by a sequence of reactions involving hydroxylation to yield 2,3,5-trihydroxytoluene followed by meta-cleavage to give 2,4,6-trioxoheptanoate; subsequent hydrolysis of this product gives acetate and pyruvate (7; D. W. Ribbons and Y. Ohta, Bacteriol. Proc., p. 9, 1970).

It was suggested that two substrate analogues, resorcinol and m-cresol, were also hydroxylated, since they too stimulate oxygen consumption when exposed to whole cell suspensions of orcinol-grown cells but not glucose-grown cells. High rates of oxygen consumption and reduced nicotinamide adenine dinucleotide (NADH) oxidation were also observed when extracts of orcinol-grown cells were incubated with resorcinol or m-cresol (Fig. 1). Furthermore, the presumed analogous products of the hydroxylation reactions for resorcinol and m-cresol (hydroxyquinol and 3-methylcatechol) were also shown to be substrates for the ring cleavage enzyme induced during growth on orcinol. Thus, it seemed possible that a nonspecific reaction sequence was induced in P. putida during growth on orcinol, which could catabolize analogous substrates to acetate and pyruvate, just as had been proposed for monohydrick phenol oxidations, hydrocarbon oxidations, or bicyclic monoterpenoid oxidations in other pseudomonads (1-3, 5-7).

This conclusion was further supported when it was found that twice crystallized orcinol hydroxylase catalyzed the oxidation of NADH by oxygen in the presence of orcinol, resorcinol, or m-cresol. The ratio of the specific activities for NADH oxidation in the presence of orcinol, resorcinol, or m-cresol were 1:0.25:0.06 for crude cell-free extracts and 1:0.19:0.06 for crystalline orcinol hydroxylase. However, detailed analysis

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FIG. 1. Oxidation of reduced nicotinamide adenine dinucleotide (NADH) by extracts of Pseudomonas putida in the presence of aromatic effectors. Each reaction mixture contained: 100 mM KH2PO4-NaOH buffer, pH 6.8 (2.8 ml); 25 mM NADH (100 μlitters); 25 mM orcinol, resorcinol, or m-cresol (50 μlitters); and cell-free extract (100 μlitters, 3.4 mg of protein). Reactions were initiated by enzyme addition, and the absorbancy at 340 nm was recorded. Temperature, 30 C. Figures appearing under the traces represent the rate of NADH oxidation (nmoles NADH oxidized per minute). Bar labeled "E340nm 0.1" represents an absorbancy change of 0.1 at 340 nm using 10-mm light path cuvettes.
of the quantitative relationships of the reactants in these oxidations revealed discrepancies from those expected for mono-oxygenase reactions (4, 8). Furthermore, it was established that, when resorcinol or m-cresol were the effectors for O₂ and NADH consumption by orcinol hydroxylase, hydrogen peroxide was a product of the reactions (8). It is now clear that resorcinol and m-cresol are able to uncouple electron transport from the hydroxylation reaction. Resorcinol is partially hydroxylated to yield hydroxyquinol; but we have not been able to detect any hydroxylated product from m-cresol (presumably 3-methylcatechol), and in this case hydrogen peroxide is formed stoichiometrically (Table 1).

Thus, our earlier conclusions, based on assays of oxygen consumption or NADH disappearance, that enzymes of the orcinol pathway could be used to transform analogous compounds to common metabolites appear to be erroneous. Indirect assays such as these, particularly for mono-oxygenase reactions, where it is now clear that uncoupling of electron transport from oxygenation is a possibility, should be interpreted with some caution. Direct evidence for the chemical transformation of the carbon substrate also needs to be provided.

### LITERATURE CITED


