2-Phenylethylamine Catabolism by *Escherichia coli* K12

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Escherichia coli K12 grows on 2-phenylethylamine as sole carbon and energy source by converting it, via phenylacetaldehyde, to phenylacetic acid. Phenylacetaldehyde was formed by the action of an inducible amine oxidase and catalase activity was increased sixfold, presumably to ensure removal of the H_2O_2 that was expected to be a product of the amine oxidation. The phenylacetaldehyde was oxidized to phenylacetic acid by an inducible NAD^+ -dependent dehydrogenase. Mutants defective in phenylacetaldehyde dehydrogenase cannot grow on 2-phenylethylamine as carbon and energy source but can still use it as a nitrogen source.

INTRODUCTION

A previously unsuspected ability of *Escherichia coli* to grow on aromatic compounds as sole carbon and energy source has been described recently (Cooper & Skinner, 1980; Burlingame & Chapman, 1983; Cooper *et al.*, 1985). It had been noted earlier that 2-phenylethylamine, the product of phenylalanine decarboxylation, could serve as a nitrogen source for *E. coli* (Scarlett & Turner, 1976) but its use as a carbon and energy source has not been reported.

Various animal tissues contain a non-specific amine oxidase that forms phenylacetaldehyde from 2-phenylethylamine (Blaschko, 1963) but little is known about 2-phenylethylamine catabolism by bacteria. *Achromobacter eurydice* forms phenylacetaldehyde from phenylpyruvic acid during phenylalanine catabolism and this phenylacetaldehyde is oxidized in an NAD^+ -dependent reaction to give phenylacetic acid (Fujioka *et al.*, 1970). Since *E. coli* K12 can grow on phenylacetic acid (Cooper *et al.*, 1985) it was possible that 2-phenylethylamine would also support growth through its conversion, via phenylacetaldehyde, to phenylacetic acid. Here we present evidence in support of this proposal.

METHODS

Bacterial strains and growth conditions. The *E. coli* HfrC strain K10, a derivative of strain K12 (Bachmann, 1972) was used. Cultures were grown aerobically at 30 °C in minimal medium 63 or Luria broth (Miller, 1972). Individual carbon sources were sterilized separately and added aseptically to give a final concentration of 5 mM unless indicated otherwise. Liquid media were solidified as required by the incorporation of 1.6% (w/v) Oxoid bacteriological agar.

Substrate oxidation. Washed-cell suspensions were prepared and oxygen consumption of cell suspensions or cell-free extracts was measured as described previously (Cooper & Skinner, 1980).

Preparation of cell-free extracts. Bacteria from 100 ml growth medium were harvested in mid-exponential phase ($OD_{680} \approx 0.3$), washed with 20 ml 0.1 M-sodium/potassium phosphate buffer pH 7.0 and finally resuspended in 4 ml of the same buffer. The cells were broken by ultrasonication and the extracts centrifuged at 120000 *g* as described previously (Donnelly & Cooper, 1981). Protein was determined by a dye-binding method (Bradford, 1976). The crude extracts contained 1–2 mg protein ml^{-1} .

Enzyme assays. 2-Phenylethylamine oxidase was assayed by measuring either the formation of phenylacetaldehyde spectrophotometrically or oxygen consumption polarographically. For a discontinuous colorimetric assay the reaction mixture contained in 4 ml: 87.5 mM-sodium/potassium phosphate buffer pH 6.1, 5 mM-2-phenylethylamine and crude extract (≈ 0.6 mg protein). At timed intervals 1 ml samples were withdrawn and added to 0.33 ml 0.1% (w/v) 2,4-dinitrophenylhydrazine.HCl in 2 M-HCl. After incubation at room temperature for 10 min, 1.67 ml 10% (w/v) NaOH was added and the absorbance at 435 nm was measured after a further 60 min. In this

system 1 μ mol of phenylacetaldehyde gave a reading of 2.5 absorbance units. For the continuous spectrophotometric assay the reaction mixture contained in a total volume of 1 ml: 95 mM-sodium/potassium phosphate buffer pH 6.0, 2 mM-semicarbazide.HCl, 1 mM-2-phenylethylamine and crude extract (≈ 0.02 mg protein). The increase in absorbance at 230 nm was monitored. The molar absorption coefficient for phenylacetaldehyde semicarbazone was $1.6 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$. For the polarographic assay the reaction mixture contained 3 mM-2-phenylethylamine and crude extract (≈ 0.15 mg protein) in 1.3 ml air-saturated 0.1 M-sodium/potassium phosphate buffer pH 6.0.

Phenylacetaldehyde dehydrogenase was assayed from the increase in absorbance at 340 nm as NAD^+ was reduced. Reaction mixtures contained in a total volume of 1.0 ml: 93 mM-sodium/potassium phosphate buffer pH 8.0, 0.75 mM- NAD^+ , 10 μ M-phenylacetaldehyde and crude extract (≈ 0.02 mg protein). A molar absorption coefficient of $6220 \text{ M}^{-1} \text{ cm}^{-1}$ was used to calculate the amount of NADH formed. The combined activities of 2-phenylethylamine oxidase and phenylacetaldehyde dehydrogenase were measured in a similar system but using buffer at pH 7.0 and with 2 mM-2-phenylethylamine instead of phenylacetaldehyde.

Catalase was assayed from the decrease in absorbance at 240 nm as H_2O_2 was decomposed (Beers & Sizer, 1952). The reaction mixture contained in a total volume of 1.0 ml: 80 mM-sodium/potassium phosphate buffer pH 7.0, 15 mM- H_2O_2 and crude extract (≈ 0.07 mg protein). A molar absorption coefficient of $40 \text{ M}^{-1} \text{ cm}^{-1}$ was used to calculate the amount of H_2O_2 consumed.

All assays were done at 30 °C.

Isolation of mutants. Cell suspensions were treated in minimal salts medium 63 with ethyl methanesulphonate as described by Miller (1972). Survivors were allowed to grow overnight at 30 °C on glucose minimal medium and 2-phenylethylamine-negative mutants were isolated after a penicillin-enrichment procedure (Miller, 1972).

Metabolism of 2-phenyl[1- ^{14}C]ethylamine. The procedures involved were as described previously for phenylacetate (Cooper *et al.*, 1985).

Chemicals. All chemicals used were the best grade commercially available. Phenylacetaldehyde was dissolved in 2% (v/v) 2-propanol to give a 1 mM solution. 2-Phenyl[1- ^{14}C]ethylamine was from Amersham.

RESULTS

Whole-cell metabolism of 2-phenylethylamine

E. coli strain K10 grew quite readily at 30 °C, but not at 37 °C, on solid medium containing 2-phenylethylamine. In liquid culture at 30 °C the mean doubling time on 2-phenylethylamine was 240 min, somewhat longer than for growth on phenylacetate (mean doubling time 180 min) or succinate (mean doubling time 150 min).

Two phenylacetate-negative mutants of strain K10, CO160 and CO164 (Cooper *et al.*, 1985), failed to grow on 2-phenylethylamine as sole carbon and energy source. However, they both grew normally when 2-phenylethylamine served as nitrogen source. 2-Phenylethylamine-grown K10 cells readily oxidized phenylacetate and 2-phenylethylamine. K10 cells grown on phenylacetate readily oxidized that compound but were unable to oxidize 2-phenylethylamine.

When the phenylacetate-negative mutant CO160 was grown on succinate + 2-phenylethylamine and cells were incubated with 2-phenyl[1- ^{14}C]ethylamine, chromatographic analysis showed that the 2-phenylethylamine disappeared with a consequential formation of phenylacetate and eventually of 2-hydroxyphenylacetate.

Metabolism of 2-phenylethylamine by cell-free extracts

2-Phenylethylamine oxidase activity. When crude extracts from 2-phenylethylamine-grown strain K10 were incubated in a oxygen monitor, gas consumption, dependent on the presence of 2-phenylethylamine, occurred at a rate of 30–40 nmol min $^{-1}$ (mg protein) $^{-1}$. A sample of the reaction mixture was reacted with 2,4-dinitrophenylhydrazine and the alkaline absorption spectrum of the 2,4-dinitrophenylhydrazone formed was identical to that given by authentic phenylacetaldehyde (λ_{max} 434 nm and 512 nm). No oxygen consumption was detected when extracts from phenylacetate- or succinate-grown cells were used. When phenylacetaldehyde was measured quantitatively as its 2,4-dinitrophenylhydrazone in a discontinuous assay its rate of formation was twice the rate of oxygen consumption obtained under equivalent conditions. In one experiment the rate of oxygen consumption was monitored for 10 min and then the amount of phenylacetaldehyde that had been formed was measured as its 2,4-dinitrophenylhydrazone.

Table 1. *Activities of enzymes involved in 2-phenylethylamine catabolism*

Details on growth of cells, preparation of extracts and enzyme assays are given in Methods. Amine oxidase was measured by the continuous spectrophotometric assay. The measurements have been made, with slight variations, on more than five occasions and representative results are shown.

Growth substrate	Specific activity [$\mu\text{mol min}^{-1} (\text{mg protein})^{-1}$]		
	Amine oxidase	Phenylacetaldehyde dehydrogenase	Catalase
2-Phenylethylamine	0.22	0.33	42.8
Phenylacetate	<0.002	<0.001	7.4
Succinate	<0.002	<0.001	7.4

The results showed that twice as much phenylacetaldehyde was formed as oxygen consumed. This is consistent with the formation of H_2O_2 by the amine oxidase reaction and its rapid decomposition by catalase:

Unlike the amine oxidases from various animal sources that can act on 2-phenylethylamine this enzyme could not be measured in a continuous assay using benzylamine as substrate (Blaschko, 1963). However, a continuous assay in which the phenylacetaldehyde formed from 2-phenylethylamine reacted with semicarbazide to form a semicarbazone was devised. The activity of the amine oxidase in cells grown on 2-phenylethylamine, phenylacetate and succinate, as measured by this assay, is given in Table 1.

Catalase activity. Since the amine oxidase reaction produces H_2O_2 it was anticipated that catalase activity might be increased during growth on 2-phenylethylamine. Table 1 shows that the normally high activity of catalase was increased six-fold during growth on 2-phenylethylamine. Whether all forms of catalase (Hassan & Fridovich, 1978; Lowen, 1984) were increased is not known.

Phenylacetaldehyde dehydrogenase activity. Phenylacetaldehyde dehydrogenase could not be detected in phenylacetate- or succinate-grown cell extracts but was readily detectable in extracts of cells grown on 2-phenylethylamine (Table 1). As noted for the enzyme from *Achromobacter eurydice* (Fujioka *et al.*, 1970) the *E. coli* enzyme was sensitive to inhibition by excess phenylacetaldehyde. A concentration of $10 \mu\text{M}$ -phenylacetaldehyde gave the highest reaction rate, the same concentration that was reported to be optimal for the *A. eurydice* enzyme.

Coupled 2-phenylethylamine oxidase and phenylacetaldehyde dehydrogenase activity. The formation of NADH through the combined action of the oxidase and dehydrogenase could also be measured. Since the amine oxidase was more active at pH 6 than at pH 7 or 8 and the dehydrogenase was more active at pH 8 than at pH 7 or 6 it was necessary to carry out this reaction at pH 7 where both enzymes showed reasonable activity. Typically, an overall rate of $140 \text{ nmol min}^{-1} (\text{mg protein})^{-1}$ was obtained. With tyramine (4-hydroxyphenylethylamine) instead of 2-phenylethylamine the rate of NADH formation was $120 \text{ nmol min}^{-1} (\text{mg protein})^{-1}$ and when dopamine (3,4-dihydroxyphenylethylamine) was used the rate was $70 \text{ nmol min}^{-1} (\text{mg protein})^{-1}$. 3-Phenylpropylamine also reacted but more slowly than 2-phenylethylamine, with a rate of $17 \text{ nmol min}^{-1} (\text{mg protein})^{-1}$. None of these compounds supported NADH formation when extracts from phenylacetate-grown cells were used.

Analysis of 2-phenylethylamine-negative mutants

Seven 2-phenylethylamine-negative, phenylacetate-positive mutants were obtained from a single mutagenesis. When they were tested on succinate plates with 2-phenylethylamine as nitrogen source five were still unable to grow but two now grew.

To determine their enzymic defects the mutants were grown overnight on 15 mM-succinate/ammonia medium and then regrown for two to three cell doublings on the same medium with 5 mM-2-phenylethylamine present to act as inducer. The cell-free extracts from the five mutants unable to utilize 2-phenylethylamine had no detectable amine oxidase or phenylacetaldehyde dehydrogenase activity. Extracts from the two mutants able to utilize 2-phenylethylamine as nitrogen source had normal amine oxidase activity but no detectable phenylacetaldehyde dehydrogenase. All seven mutants showed the expected catalase activity. When the ability of whole cells to take up and metabolize 2-phenyl[1-¹⁴C]ethylamine was tested all five mutants unable to grow with 2-phenylethylamine showed no uptake of radioactivity but the two phenylacetaldehyde dehydrogenase-negative mutants took up the labelled compound.

When one of the phenylacetaldehyde dehydrogenase-negative mutants, strain CO184, was grown on succinate with 2-phenylethylamine, the culture supernatant contained a carbonyl compound whose alkaline 2,4-dinitrophenylhydrazone (λ_{max} 434 nm and 512 nm) was identical to that obtained with authentic phenylacetaldehyde.

DISCUSSION

The results presented here for whole cells, cell-free extracts and mutants are all consistent with the catabolism of 2-phenylethylamine to phenylacetate by the sequential action of an amine oxidase and a phenylacetaldehyde dehydrogenase.

Two distinct classes of amine oxidase are described in the literature. One class (EC 1.4.3.4) contains flavin and the other (EC 1.4.3.6) contains copper. Very recently some amine oxidases in the latter class have been found to contain pyrroloquinoline quinone (PQQ) as a cofactor (Duine *et al.*, 1986). It is not yet clear which class the *E. coli* amine oxidase belongs to. As *E. coli* is believed to be unable to make PQQ (Duine *et al.*, 1986), it seems more likely to be a flavin-containing enzyme.

The operation of the amine oxidase means that a molar equivalent of potentially lethal H₂O₂ is formed for each 2-phenylethylamine catabolized. Catalase activity is normally high in *E. coli* (Yoshpe-Purer *et al.*, 1977; Hassan & Fridovich, 1978) and appears to be inducible by exogenously supplied H₂O₂. However, induction by endogenously produced H₂O₂, as reported here, does not seem to have been described previously for *E. coli* although it has been observed during amine utilization by an *Arthrobacter* species (Levering *et al.*, 1981) and by *Candida boidinii* (Haywood & Large, 1981). It seems likely that *E. coli* mutants devoid of catalase would fail to grow on 2-phenylethylamine, but since there are at least two genetically distinct forms of catalase in *E. coli* (Loewen, 1984), mutants totally devoid of catalase are unlikely to be found amongst single-gene 2-phenylethylamine-negative mutants.

The ability to form NADH in the coupled reaction with tyramine, dopamine and 3-phenylpropylamine as substrates was inducible by growth on 2-phenylethylamine. This suggests that both the amine oxidase and phenylacetaldehyde dehydrogenase are not specific, but the purified enzymes are needed for a proper assessment of their substrate specificities. Further characterization of these enzymes is under way.

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