Genetic and Structural Evidence for the Presence of Propanediol Oxidoreductase Isoenzymes in *Escherichia coli*

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The synthesis of propanediol oxidoreductase, an enzyme permitting the anaerobic metabolism of fucose and rhamnose, has been described as being controlled by the *prd* locus closely linked to the *fuc* locus in wild-type cells of *Escherichia coli*. However, strain AA-787, deleted in the *fuc* and *prd* loci, grew anaerobically on rhamnose, displaying propanediol oxidoreductase activity. From the deleted strain we derived a constitutive producer of propanediol oxidoreductase able to grow on 1,2-propanediol by oxidizing the diol to lactaldehyde which was further metabolized to lactate. Transduction experiments showed that this ability to use propanediol was closely linked to the *rha* locus. Peptide mapping of fucose- and rhamnose-induced propanediol oxidoreductase of wild-type cells established structural differences between the two enzymes, indicating two structural genes, one for each sugar metabolizing system.

INTRODUCTION

Propanediol oxidoreductase (EC 1.1.1.77) is an enzyme of the fucose and rhamnose fermentation pathways in *Escherichia coli* (Cocks *et al.*, 1974; Boronat & Aguilar, 1979). Both pathways converge after the cleavage of fuculose-1-phosphate and rhamnulose-1-phosphate, yielding the same two products, dihydroxyacetone phosphate and L-lactaldehyde. The oxidoreductase converts the L-lactaldehyde formed into L-1,2-propanediol which is excreted into the medium as a fermentation product (Cocks *et al.*, 1974).

As expected, propanediol oxidoreductase is induced by anaerobic growth of wild-type *E. coli* on fucose (Cocks *et al.*, 1974) and rhamnose (Boronat & Aguilar, 1979). However, mutants of the wild-type strain may be isolated in which the production of propanediol oxidoreductase is constitutive. In these, the enzyme acts by converting L-1,2-propanediol to lactaldehyde, thus permitting growth of these cells on L-1,2-propanediol (Sridhara *et al.*, 1969). Acquisition of this catabolic pathway depends on a genetic alteration of the dissimilatory system for L-fucose, on which most of the mutants are no longer able to grow (Hacking & Lin, 1976).

This constitutive production of propanediol oxidoreductase, phenotypically expressed as the ability to grow on L-1,2-propanediol, has been located by transduction very close to the *relA* gene (Russell, 1973) and has been shown to co-transduce with 100% frequency with the *fuc* locus (Hacking *et al.*, 1978).

The induction of propanediol oxidoreductase by anaerobic growth on rhamnose (Boronat & Aguilar, 1979), the observation of differences between the fucose and rhamnose enzyme induction (Boronat & Aguilar, 1981*b*) and the description of two independent sets of enzymes for the aerobic metabolism of fucose (Green & Cohen, 1956; Ghalambor & Heath, 1962; Heath & Ghalambor, 1962) and rhamnose (Takagi & Sawada 1964*a,b*; Chiu & Feingold, 1969) which are coded by genes located 27 min apart in the *E. coli* chromosome, led us to search for a gene for propanediol oxidoreductase linked to the *rha* operon.

METHODS

Bacteria and phage. The wild-type strain was a derivative of *E. coli* K12, strain E-15 (Bachmann, 1972) and here referred to as strain 1. Strain 3 was derived from strain 1 as a propanediol oxidoreductase mutant after ethyl methanesulphonate mutagenesis (Sridhara *et al.*, 1969). These two strains were from the laboratory of E. C. C. Lin, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA 02115, USA. A mutant of *E. coli* K12, strain AA-787, containing a large deletion encompassing *argA*, *fuc* and *relA* (Atherly, 1979) was kindly provided by A. G. Atherly, Department of Genetics, Iowa State University, Ames, IA 50011, USA. This strain was also *argH*, *thr*, *leu*, *his*, *thi* and *thyA*. Strain DF-903 containing a deletion from *rha* to *pskA* (Daldal & Fraenkel, 1981) was given to us by D. G. Fraenkel, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA 02115, USA. Transduction with phage P1 was carried out by the method of Miller (1972).

Chemicals. DL-1,2-propanediol was obtained from Merck, Darmstadt, F.R.G. and purified by distillation. L-Lactaldehyde was prepared in our laboratory by the procedure of Boronat & Aguilar (1979). L-Fucose, L-rhamnose and NADH were obtained from Sigma, and casein acid hydrolysate was from Difco. The enzyme α -chymotrypsin from beef pancreas was obtained from Worthington, Freehold, NJ, USA. Gel electrophoresis materials were from Bio-Rad. All other materials were reagent grade and obtained from commercial sources.

Growth of cells. Cells were grown as indicated by Boronat & Aguilar (1979). Strain AA-787 was grown in the same media supplemented with amino acids at 10 to 50 $\mu\text{g ml}^{-1}$ depending upon their ratio in proteins (or by adding 0.2% casein acid hydrolysate), thiamin (1 $\mu\text{g ml}^{-1}$) and thymidine (4 $\mu\text{g ml}^{-1}$).

Preparation of cell extracts and enzyme assay. Cell extracts were prepared and enzymes assayed as described previously (Boronat & Aguilar, 1981*b*). One unit of enzyme activity was defined as the amount of enzyme that converted 1 μmol substrate min^{-1} .

Concentration of protein in cell extracts was determined by the Lowry method, using bovine serum albumin as standard.

Immunological techniques. Antisera against propanediol oxidoreductase were obtained as described previously (Boronat & Aguilar, 1981*b*). Double immunodiffusion and quantitative immunoelectrophoresis were performed as described by Ouchterlony (1953) and Laurell (1966), respectively.

Peptide mapping. For peptide mapping, propanediol oxidoreductase from strain 1 grown on fucose or rhamnose was purified as described previously (Boronat & Aguilar, 1979). In order to eliminate some minor contaminants, the enzyme preparations were further purified by electrophoresis according to Laemmli (1970). After a brief staining, the band corresponding to propanediol oxidoreductase was sliced from the gel and electrophoretically eluted into a dialysis bag using 0.1% (w/v) SDS in 2.5 mM-Tris/glycine (pH 8.3) as buffer. The protein content of the bag was precipitated with nine volumes of cold acetone, resuspended in 100 mM-ammonium bicarbonate and dialysed overnight against the same buffer. The samples were lyophilized in 1 ml conical glass vials and taken up in 0.05% (w/v) SDS, 100 mM-ammonium bicarbonate (pH 8.0) at a protein concentration of 1 mg ml^{-1} .

The protein was then digested with the chymotrypsin under the conditions indicated below. A sample of 1 nmol protein digest was spotted on a cellulose thin-layer plate that had previously been washed overnight by ascending chromatography in butanol/pyridine/acetic acid/water (50:33:1:40, by vol.). Electrophoresis in the first dimension was carried out in a Varsol cooled apparatus (Whittaker & Moss, 1981), using pyridine/acetic acid/water (1:10:89, by vol.) as buffer and applying 900 V for 30 min. After drying, the plates were kept at room temperature for 10 min and then chromatographed at right angles to the first dimension in the butanol/pyridine/acetic acid/water solvent. The dried, cooled plates were sprayed with 0.1% (w/v) fluorescamine in acetone, and the peptides visualized under long-wavelength UV light.

RESULTS

Growth properties of strain AA-787

As the *fuc* and *prd* loci are deleted in strain AA-787 it did not grow on fucose either aerobically or anaerobically. This strain, as expected, grew aerobically on rhamnose with a doubling time of 110 min and, in spite of the lack of the *prd* locus, also grew anaerobically on rhamnose with a doubling time of 260 min. The yields of aerobic and anaerobic cultures of strain AA-787 were similar to those obtained in the same conditions with wild-type *E. coli*.

Propanediol oxidoreductase in strain AA-787

Strain AA-787 displayed a very low propanediol oxidoreductase activity when grown aerobically on casein acid hydrolysate or rhamnose. However, this activity was induced five- to sixfold when it was grown on rhamnose anaerobically (Table 1).

Table 1. Activity of propanediol oxidoreductase in crude extracts of strains AA-787 and JA-101 grown under different conditions

Carbon source	Growth condition	Enzyme activity [units (mg protein) ⁻¹]	
		AA-787	JA-101
Casein hydrolysate	Aerobic	0.10	0.48
Rhamnose	Aerobic	0.08	0.44
Rhamnose	Anaerobic	0.50	0.61

Fig. 1.

Fig. 1. Immunological identity between propanediol oxidoreductase of strain 1, strain 3 and strain AA-787. Wells in the Ouchterlony immunodiffusion plates contained 25 μ l cell extracts of: (a) strain 1 grown anaerobically on fucose (30 μ g protein), (b) and (e) strain 3 grown aerobically on casein hydrolysate (6 μ g protein), (c) strain 1 grown anaerobically on rhamnose (30 μ g protein) and (d) and (f) strain AA-787 grown anaerobically on rhamnose (30 μ g protein). The centre well contained 25 μ l antiserum.

Fig. 2.

Fig. 2. Immuno-electrophoretic quantification of propanediol oxidoreductase. The Laurell rockets were obtained by applying to a gel containing 1.5% antiserum, 25 μ g protein in 10 μ l cell extract of: (1) strain AA-787 grown aerobically on casein hydrolysate, (2) strain AA-787 grown anaerobically on rhamnose and (3) strain JA-101 grown aerobically on casein hydrolysate.

Propanediol oxidoreductase induced in strain AA-787 appeared identical to the wild-type enzyme by double immunodiffusion against enzyme antibodies (Fig. 1). This permitted us to quantify the amount of enzyme by Laurell immunoelectrophoresis of crude extracts. Under our conditions, the length of the Laurell rocket was proportional to the amount of enzyme over a wide range of concentrations. The enzymic protein was almost non-existent in strain AA-787 grown under non-inducing conditions, as shown by the absence of a rocket when such extracts were analysed. In agreement with the presence of enzymic activity in extracts of cells grown under inducing conditions, the rocket was apparent in the immunoelectrophoresis of such extracts (Fig. 2).

A propanediol oxidoreductase constitutive mutant of strain AA-787

A constitutive producer of propanediol oxidoreductase was derived from strain AA-787 by isolating mutants able to grow on 1,2-propanediol (Sridhara *et al.*, 1969). Selection was performed on DL-1,2-propanediol plates inoculated with an ethyl methanesulphonate

Fig. 3. Peptide mapping of purified propanediol oxidoreductase from strain 1 grown anaerobically on (a) fucose or (b) rhamnose. Arrows indicate spots present on one map but not on the other.

mutagenized population as described by Lin *et al.* (1962). A clone (strain JA-101) was chosen for its good growth on these plates and was subsequently restreaked four times on the same medium. This strain grew aerobically on propanediol as a sole source of carbon and energy with a doubling time of 95 min and also grew on rhamnose aerobically and anaerobically.

Extracts of strain JA-101 exhibited propanediol oxidoreductase activity when the cells were grown under inducing as well as under non-inducing conditions (Table 1). Furthermore, this enzymic activity could be correlated with the production of propanediol oxidoreductase protein as measured by the length of the corresponding Laurell rocket (Fig. 2).

The ability of strain JA-101 to grow on propanediol co-transduced with the *rha* locus was shown by an experiment in which strain DF-903 carrying a deletion in the *rha* locus was transduced

with P1 phage grown on strain JA-101. An estimated 80% of the transductants that recovered the ability to grow on rhamnose also acquired the ability to grow on propanediol. The frequency of mutation to the ability to grow on propanediol as a result of the constitutive production of the propanediol oxidoreductase linked to the *fuc* locus was far below the number of transductants plated.

Structural differences between fucose and rhamnose induced propanediol oxidoreductase

Propanediol oxidoreductase of strain 1 grown anaerobically on fucose or rhamnose was purified as indicated in Methods. The purified proteins were digested with α -chymotrypsin in a propanediol oxidoreductase to chymotrypsin ratio of 100:1 (w/w) by incubating the mixture at 37 °C for 10 h with gentle stirring. Before being electrophoresed and chromatographed as indicated in Methods, the samples were concentrated 10-fold using a stream of dry nitrogen at 40 °C. The peptide maps obtained are shown in Fig. 3. The arrows indicate the main differences between the spot patterns obtained with the enzymes induced by fucose and rhamnose.

DISCUSSION

As discussed by Boronat & Aguilar (1981*b*) there are two possibilities for the regulation of propanediol oxidoreductase during fucose and rhamnose fermentation. One is that both systems use the same propanediol oxidoreductase linked to the *fuc* locus (Bachman & Low, 1980), although its expression is regulated in a different way; the other is that each system has its own propanediol oxidoreductase (coded by highly similar genes since their products cannot be easily distinguished).

If both sugars were fermented by the action of a single propanediol oxidoreductase specified by a gene linked to *fuc*, the lack of this enzyme produced by the deletion of the *prd* and *fuc* loci would necessarily result in the inability of the deletion mutants to ferment either fucose or rhamnose. Neither genetic nor biochemical complementation would be possible in such mutants. Our results with strain AA-787 indicate that this is not the case; there is another means of obtaining propanediol oxidoreductase expression in these cells. The possible absence of propanediol oxidoreductase activity as a consequence of inhibition of enzyme activity by a biochemical regulatory mechanism was eliminated by the immunological quantification of the enzyme protein.

The results obtained with strain AA-787 and its derivative strain JA-101 confirm the presence of a regulatory gene, besides the one linked to *fuc*, acting on the expression of the oxidoreductase. The close linkage of this gene for oxidoreductase expression to *rha* was ascertained not only by the rhamnose induction of the activity but also by the co-transduction of constitutive propanediol oxidoreductase synthesis in strain JA-101 with the *rha* locus.

So far, no functional or structural difference between the fucose- and rhamnose-induced propanediol oxidoreductase has been described, but there are several reports of enzymes, indistinguishable by their functional parameters, that actually show differences in peptide mapping or amino acid sequencing (Koo & Adams, 1974; Kikuchi & Gorini, 1975; Boronat & Aguilar, 1981*a*). Here we show structural differences as indicated by the different patterns of spots obtained in the two-dimensional peptide mapping of the purified enzymes induced by fucose or rhamnose. This is in accord with the existence of a regulatory system for propanediol oxidoreductase expression different from the one specified by a gene linked to *fuc*. It would also be compatible with the presence of two different structural genes of undetermined location in addition to the regulatory genes described – one close to the *fuc* locus, the other close to the *rha* locus.

The two similar sets of genes for the parallel metabolism of fucose and rhamnose lie 27 min apart on the map of the *E. coli* chromosome. According to the hypothesis of Riley & Anilionis (1978) concerning the mechanism of evolution of the bacterial genome, these two sets of genes might be derived by duplication of the entire chromosome. Although the propanediol oxidoreductase genetic system is not included in the sets of genes described, the existence of a similar duplication for the oxidoreductase would not be surprising (Zipkas & Riley, 1975). One of the

two propanediol oxidoreductase genes may be lost or silent, although our results indicate that this is not the case.

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