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The Utilisation of *D-Galactonate and D-2-Oxo-3-Deoxygalactonate by *Escherichia coli* K-12

Biochemical and Genetical Studies

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Abstract. 1. *Escherichia coli* K-12 mutants unable to grow on D-galactonate have been isolated and found to be defective in either galactonate dehydratase, 2-oxo-3-deoxygalactonate 6-phosphate aldolase or devoid of both of these enzymes and of 2-oxo-3-deoxygalactonate kinase.

2. 2-Oxo-3-deoxygalactonate kinase and 2-oxo-3-deoxygalactonate 6-phosphate aldolase are still induced by galactonate in mutants lacking galactonate dehydratase, suggesting that galactonate rather than a catabolic product of galactonate is the inducer of the galactonate catabolic enzymes. Synthesis of the enzymes is subject to glucose catabolite repression.

3. Mutants defective in 2-oxo-3-deoxygalactonate 6-phosphate aldolase accumulate 2-oxo-3-deoxygalactonate 6-phosphate when exposed to galactonate and this compound causes general growth inhibition.

4. Secondary mutants that no longer show this inhibition fail to make 2-oxo-3-deoxygalactonate 6-phosphate due to additional defects in galactonate transport, galactonate dehydratase, 2-oxo-3-deoxygalactonate kinase or a putative promoter mutation that prevents formation of these enzymes.

5. A spontaneous mutant capable of growth on 2-oxo-3-deoxygalactonate has been isolated. It has two genetically distinct mutations. One permits constitutive formation of the galactonate catabolic enzymes and the other allows the uptake of 2-oxo-3-deoxygalactonate. Neither mutation on its own permitted growth on 2-oxo-3-deoxygalactonate.

6. Genes specifying the various galactonate catabolic enzymes have been located at min 81.7 on the *E. coli* K-12 linkage map and probably constitute an operon. The gene sequence in this region was shown to be: *pyr*E *uhp dgo dna*A.

Key words: *E. coli* K-12 – Galactonate – 2-Oxo-3-deoxygalactonate – Genetic mapping.

The aldonic sugar acids have no functional groups that render them readily detectable and this may account for the dearth of information on their biological occurrence. They are known as intermediates in bacterial sugar catabolism (Scott and Cohen, 1953; De Ley and Doudoroff, 1957; Ashwell et al., 1958) and more recently galactonate has been identified as a significant product of galactose catabolism in both normal and galactosemic humans (Bergren et al., 1972). It is thus possible that galactonate is more common than is presently supposed and this could account for the very ready growth of the enteric bacteria on galactonate that we reported recently (Deacon and Cooper, 1977).

The pathway that we proposed for galactonate utilisation involved the conversion of galactonate to

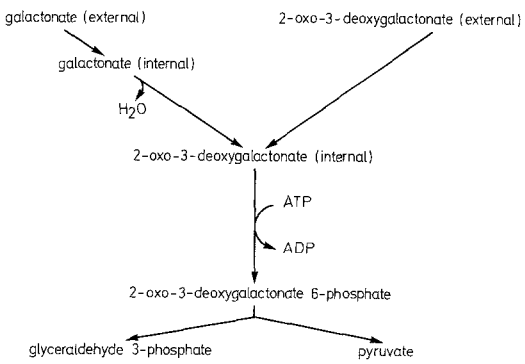

Fig. 1. A schematic representation of the pathways for galactonate and 2-oxo-3-deoxygalactonate catabolism by *Escherichia coli* K-12

* Where no steroisomeric form is indicated, the sugars are of the D series

Abbreviations. ODGalO kinase = 2-oxo-3-deoxygalactonate kinase; ODGalOP aldolase = 2-oxo-3-deoxygalactonate 6-phosphate aldolase

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pyruvate and glyceraldehyde 3-phosphate by the sequential action of galactonate dehydratase (EC 4.2.1.6), ODGalO kinase (EC 2.7.1.58) and ODGalOP aldolase (EC 4.1.2.21) (Fig. 1). The formation of these enzymes appeared to be tightly regulated since they were present only during growth in the presence of galactonate.

We have now obtained mutants that are unable to grow on galactonate and used these mutants to investigate further the catabolic pathway for galactonate. Additional information on the regulation of galactonate catabolism has been obtained by a study of the growth of *Escherichia coli* K-12 on 2-oxo-3-deoxygalactonate. This intermediate in galactonate catabolism is not a carbon source for the growth of wild-type *Escherichia coli* K-12 strains but spontaneous mutants that can utilise 2-oxo-3-deoxy-galactonate have been obtained.

Materials and Methods

Bacterial Strains and Growth Conditions. The *Escherichia coli* K-12 strains used in the course of this work are described in Table 1. They were grown aerobically at 37° C in appropriately supplemented minimal medium 63 (Miller, 1972) with galactonate (10 mM) or glycerol (20 mM) as carbon source. Liquid media were solidified as appropriate by the incorporation of 1.6% Oxoid bacteriological agar. Stocks were maintained on nutrient agar slopes.

Genetic Procedures. The genetic procedures for conjugation and for transduction (using phage P1 *vir*) were carried out as described by Miller (1972).

Cell-Free Extracts. For the preparation of cell-free extracts bacteria from 100 ml of medium were harvested in the late logarithmic phase of growth, suspended in 4 ml of 50 mM imidazole-HCl-0.5 mM dithiothreitol buffer pH 7.0 and disrupted by exposure to ultrasonic oscillations in an MSE 100-W ultrasonic disintegrator (operating at 8 μm peak-to-peak amplitude) at 0° C for 1 min. The suspensions were then centrifuged at 25000 × g for 15 min at 4° C to remove cell debris.

Enzyme Assays. For assays involving measurement of $NADH_2$ oxidation the crude supernatant solutions were further centrifuged at 150000 × g for 1.5 h at 4° C to remove the particulate $NADH_2$ oxidase activity. Soluble protein was measured by the biuret method (Gornall et al., 1949) using crystalline bovine serum albumin as the standard. Galactonate dehydratase and ODGalOP aldolase were assayed as described previously (Deacon and Cooper, 1977). The ODGalO kinase assay described previously (Deacon and Cooper, 1977) was slightly modified to reduce the blank rate due to ATPase activity. For this the Tris-HCl buffer pH 7.5 was replaced by Na-K phosphate buffer, pH 6.8 (100 μmol) and NaF (100 μmol) was also included. The uptake of radioactive sugars was followed in a reaction mixture that contained in 1 ml: minimal medium 63 (0.8 ml), glycerol (50 μmol), bacterial cell suspension (1.5 mg dry weight) and radioactive sugar (1 μCi; 0.25 μmol). Samples (0.1 ml) were removed at known times and the cells separated rapidly by membrane filtration. The cells were washed on the filters with 5 ml of minimal medium 63 and then the filter and cell pellets dissolved in Bray's fluid (Bray, 1960).

The radioactivity of the samples was measured in a liquid scintillation counter.

Isolation of Mutants. To select galactonate-negative mutants, cultures of strain K10 were mutagenised in minimal medium 63 with

Table 1. Characteristics of the *Escherichia coli* K-12 strains used

Strain	Mating type	Genotype	Source or reference
K10	HfrC	*thi(?)*	F. C. Neidhardt
PA309	F^-	*thi, argHBCE, his, leu, thr, trp, rpsL, lacY, malA, xyl, ara, mtl, gal, tonA*	R. H. Walmsley
JM452	F^-	*pyrE, pro, metB, his, cysI, mtl, fpk, uhp, glpT*	M. C. Jones-Mortimer
AB2297	Hfr	*ilv, pur, xyl*	H. L. Kornberg
KL228	Hfr	*thi, leu, lacYorZ, gal*	B. J. Bachmann
CO100	HfrC	*thi(?), dgoA*	this paper
CO101	HfrC	*thi(?), dgoD*	this paper
CO102	HfrC	*thi(?), dgoP*	this paper
CO103	HfrC	*thi(?), dgoR, odgR(?)*	this paper
CO106	HfrC	*thi(?), dgoR, dgoA, odgR(?)*	this paper
CO109	HfrC	*thi(?), dgoR, dgoA, odgR(?), odgT*	this paper
CO110	HfrC	*thi(?), dgoR, dgoA, dgoK, odgR(?)*	this paper
CO113	HfrC	*thi(?), dgoR, dgoA, dgoT, odgR(?)*	this paper
CO117	HfrC	*thi(?), dgoR, dgoA, dgoP, odgR(?)*	this paper
CO504	F^-	*pro, metB, his, cysI, mtl, fpk, uhp, glpT*	this paper (Table 3)

The symbol *dgo* (D-galactonate) has been chosen for the genes specifying the galactonate catabolic enzymes. The symbols used are as follows: galactonate transport, *dgoT*; galactonate dehydratase, *dgoD*; 2-oxo-3-deoxygalactonate kinase, *dgoK*; 2-oxo-3-deoxygalactonate 6-phosphate aldolase, *dgoA*; a regulator gene controlling production of these enzymes, *dgoR*; a promoter locus, *dgoP*. The symbol *odg* (2-oxo-3-deoxgalactonate) has been chosen for the genes uniquely involved in 2-oxo-3-deoxygalactonate utilisation: a regulator gene permitting uptake of 2-oxo-3-deoxygalactonate, *odgR*; 2-oxo-3-deoxygalactonate transport, *odgT*

ethylmethanesulphonate as described by Miller (1972). Survivors were allowed to grow overnight at 37° C in minimal medium 63 supplemented with 10 mM glucose. Samples of these cultures were then treated with penicillin (Miller, 1972) in the presence of galactonate. The surviving cells were harvested and spread onto glucose plates. The colonies obtained were replicated onto galactonate plates to identify mutants unable to grow on galactonate.

Preparation of Radioactive Galactonate and 2-Oxo-3-Deoxygalactonate. To prepare [1-^{14}C]-galactonate, galactose dehydrogenase (EC 1.1.1.48) (40 µg) was added to a reaction mixture that contained, in 3 ml: Tris · HCl buffer pH 8.4 (300 µmol), NAD (1.5 µmol) and [1-^{14}C]-galactose (30 µCi; 0.5 µmol). The progress of the reaction was monitored at 340 nm in a recording spectrophotometer until $NADH_2$ production reached a maximum (40 – 50 min). The preparation was analysed by descending chromatography on Whatman No. 1 paper using acetone: water (85:15), concentrated ammonia: methanol: water (1:6:3), and *n*-propanol: formic acid: water (6:3:1) as solvents. After autoradiography, the appropriate areas of the chromatograms were cut out and their radioactivity was determined by liquid scintillation counting. At least 97% of the galactose had been used up and two new compounds that corresponded to galactonate and galactonolactone were produced. The [1-^{14}C]-galactonate could be converted into [1-^{14}C] 2-oxo-3-deoxygalactonate by the action of galactonate dehydratase. Production of 2-oxo-3-deoxygalactonate was monitored using the thiobarbituric acid assay (Weissbach and Hurwitz, 1959). Chromatographic analysis using the solvents described above showed that the only significant radioactive compound present was 2-oxo-3-deoxygalactonate. Such solutions were used as the source of radioactive galactonate or 2-oxo-3-deoxygalactonate without any further purification.

Estimation of 2-Oxo-3-Deoxygalactonate 6-Phosphate. 2-Oxo-3-deoxygalactonate 6-phosphate was estimated spectrophotometrically in an assay system containing in 1 ml: Tris · HCl buffer pH 7.5 (100 µmol), $MgCl_2$ (5 µmol), $NADH_2$ (0.15 µmol), crystalline lactate dehydrogenase (5 µg), partially purified ODGalOP aldolase (Deacon and Cooper, 1977) (0.02 unit) and sample (0.1 – 0.2 ml). The pyruvate formed by the action of ODGalOP aldolase was converted into lactate and the concomitant decrease in $NADH_2$ was monitored at 340 nm.

Sources of Biochemicals. Biochemicals and crystalline lactate dehydrogenase and pyruvate kinase were purchased from Boehringer (London) Ltd. Galactose dehydrogenase and galactonolactone were purchased from Sigma (London) Ltd. Galactonolactone was converted into sodium galactonate by titration with sodium hydroxide. 2-Oxo-3-deoxygalactonate was prepared enzymically from galactonate as described by DeLey and Doudoroff (1957) but using galactonate-grown *E. coli* K-12 as the source of galactonate dehydratase.

Results

Analysis of Galactonate-Negative Mutants

The galactonate-negative mutants isolated as described in the "Materials and Methods" section grew at wild-type rates with glycerol, lactate, glucose and gluconate as carbon sources, suggesting that the central pathways of glycolysis and gluconeogenesis were unaffected in these mutants. Since wild-type cells formed galactonate dehydratase, ODGalO kinase and ODGalOP aldolase only during growth in the presence of galactonate (Deacon and Cooper, 1977) it was necessary to grow the mutants in the presence of galactonate to determine whether these enzymes were absent. When the galactonate-negative mutants were tested for growth on plates containing 20 mM glycerol plus 5 mM galactonate some of the mutants failed to grow, suggesting that an inhibitor that prevented growth on glycerol was being produced from galactonate by some of the mutants. This inhibitory effect appeared to be general since addition of galactonate to such galactonate-sensitive mutants growing on glucose, lactate or succinate also caused growth inhibition.

When those mutants that were not inhibited during growth on glycerol plus galactonate were so grown and cell-free extracts analysed, two classes of mutants could be distinguished. The first class, as exemplified by strain CO 101, had no detectable galactonate dehydratase but showed normal activities of ODGalO kinase and ODGalOP aldolase. The second class, as exemplified by strain CO102, had no detectable activity of any of the galactonate catabolic enzymes.

The enzymic defect in the galactonate-sensitive mutants such as strain CO 100 could not be measured directly since they could not be grown in the presence of galactonate. When galactonate (5 mM final concentration) was added to a culture of CO 100 growing logarithmically on glycerol growth was rapidly arrested. Three hours after addition of the galactonate the cells (47 mg dry weight) were harvested and extracted for 3 min with 1 ml of boiling water. The cell-extract and the growth medium were then assayed for 2-oxo-3-deoxygalactonate 6-phosphate and 0.9 µmol per ml was present in the cell-extract and 0.19 µmol per ml was present in the growth medium. On the assumption that the dry weight of the cells is 20% of the wet weight the 2-oxo-3-deoxygalactonate 6-phosphate is present in the cells at a concentration of 4.8 mM. When the total 2-oxo-3-deoxyhexose content of the samples was measured with the thiobarbituric acid reagent (Weissbach and Hurwitz, 1959) the medium concentration was 2.1 µmol per ml and the cell extract contained 2.4 µmol per ml. This suggested that a considerable amount of the non-phosphorylated 2-oxo-3-deoxygalactonate was also present. When the wild-type strain K10 was treated similarly, or when strain CO100 was grown in the absence of galactonate, there was no 2-oxo-3-deoxygalactonate 6-phosphate detectable in either the cell-extract or growth medium. Thus although the evidence is indirect these findings suggest that ODGalOP aldolase is the defective enzyme in strain CO100.

So far at least 30 independent galactonate-negative mutants have been analysed and no mutant defective only in ODGalO kinase has yet been found. However, an ODGalO kinase-negative mutant has been obtained

Table 2. Glucose repression of galactonate catabolic enzymes

Growth substrate	Specific activity		
	Galactonate dehydratase	ODGalO kinase	ODGalOP aldolase
Galactonate	0.094	0.234	0.302
Galactonate plus glucose	0.008	0.018	0.023

Strain K10 was grown overnight on 10 mM glucose and then allowed to grow for 3 generations on the substrates indicated, each at 10 mM concentration. Specific activity is defined as μmol substrate transformed min^{-1} mg $protein^{-1}$

from a strain already defective in ODGalOP aldolase (see later) and this mutant, as anticipated, was unable to produce 2-oxo-3-deoxygalactonate 6-phosphate.

Glucose Repression of the Galactonate Catabolic Enzymes

Since galactonate was as good a carbon source for growth as was glucose (Deacon and Cooper, 1977) it was of interest to see whether growth in the presence of glucose repressed the formation of the galactonate catabolic enzymes. Table 2 shows that growth on a mixture of 10 mM glucose and 10 mM galactonate caused a 90% reduction in the activity of galactonate dehydratase, ODGalO kinase and ODGalOP aldolase. That glucose did not totally repress the formation of the galactonate catabolic enzymes was confirmed by the production of lethal amounts of 2-oxo-3-deoxygalactonate 6-phosphate when galactonate was added to the ODGalOP aldolase-negative mutant CO100 growing on glucose.

Growth on 2-Oxo-3-Deoxygalactonate

The dehydratases involved in the catabolism of various aldonic sugar acids appear to catalyse irreversible dehydrations (Ashwell, 1962; Meloche and Wood, 1966) and the dehydration of galatonate would likewise be expected to be irreversible. This means that if exogenous 2-oxo-3-deoxygalactonate catabolism involves the enzymes of galactonate catabolism, and if, as suggested in the preceeding section, galactonate alone is the inducer of all the galactonate catabolic enzymes, *Escherichia coli* K-12 strains should not be able to grow 2-oxo-3-deoxygalactonate because they would not be able to make the inducer for the necessary catabolic enzymes. When *E. coli* strain K10 was spread onto solid media with 5 mM 2-oxo-3-deoxygalactonate as sole carbon and energy source it was indeed unable to grow. However, after continued incubation at 37° C for 7 – 10 days a small number of colonies appeared on the plates. When these were picked off, purified by repeated single colony isolation and then grown on glycerol minimal medium the galactonate catabolic enzymes were present at high activity, suggesting that in these mutants the enzymes were now formed constitutively.

One such constitutive mutant, strain CO103, was used for the isolation of galactonate-negative mutants as described in the Materials and Methods section for wild-type cells. As with the mutants obtained from wild-type cells some of the galactonate-negative mutants were inhibited by the addition of galactonate (and in this case by 2-oxo-3-deoxygalactonate) during growth on other carbon sources such as glycerol. Such mutants, as exemplified by strain CO106, were shown to accumulate 2-oxo-3-deoxygalactonate 6-phosphate and 2-oxo-3-deoxygalactonate when exposed to galactonate, as described in the previous section for strain CO100. However, the constitutive production of the galactonate catabolic enzymes by strain CO103 meant that analysis of cell-free extracts prepared from glycerol-grown galactonate-negative mutants would indicate the defective enzyme without recourse to growth in the presence of galactonate. By this means it was shown that the galactonate-sensitive mutants were defective in ODGalOP aldolase. As with the wild-type strains two other classes of galactonate-negative mutants were obtained. The first class were defective in galactonate dehydratase and the second class were defective in all of the galactonate catabolic enzymes.

Use of ODGalOP Aldolase-Negative Mutants for the Selection of Additional Defects in Galactonate Catabolism

It was possible to use strain CO106, an ODGalOP aldolase-negative mutant obtained from the 2-oxo-3-deoxygalactonate-positive strain CO103, to obtain ODGalO kinase-negative, galactonate transport-negative, and putative 2-oxo-3-deoxygalactonate transport-negative mutants by the selection of secondary mutants that were resistant to the inhibitory effect of added galactonate or 2-oxo-3-deoxygalactonate. Such resistance could be due to either a mutation that conferred insensitivity to inhibition by 2-oxo-3-deoxygalactonate 6-phosphate or to mutations causing additional defects in galactonate or 2-oxo-3-deoxygalactonate catabolism thus preventing the formation of 2-oxo-3-deoxygalactonate 6-phosphate.

When strain CO 106 was spread onto 20 mM glycerol plus 5 mM 2-oxo-3-deoxygalactonate plates and incubated at 37° C for 36 h a very large number of

Table 3. Order of the *pyr*E, *uhp* and *dgo*D loci

P1 donor	+		+		*dgo*D	
(recipient)	*pyr*E		*uhp*		+	
Percentage of PyrE$^+$ transductants that are:						
Uhp$^+$	DgoD$^+$	Uhp$^+$	DgoD$^-$	Uhp$^-$	DgoD$^+$	Uhp$^-$	DgoD$^-$
36		11		52		1	

resistant colonies appeared on the plate. Some of these were picked off and tested on 20 mM glycerol plus 5 mM galactonate plates and about 50% were also now resistant to galactonate. When approximately 20 mutants resistant to both 2-oxo-3-deoxygalactonate and galactonate were tested for ODGalO kinase all were found to be defective. However, further assays showed that most were also lacking galactonate dehydratase. Nevertheless a few, as exemplified by strain CO110, had normal activity of galactonate dehydratase indicating that the absence of ODGalO kinase on its own could lead to resistance to killing by galactonate or 2-oxo-3-deoxygalactonate in the ODGalOP aldolase-negative strains. When strain CO110 was grown on glycerol and exposed to galactonate as described earlier for strains CO100 and CO106 there was no growth inhibition and no 2-oxo-3-deoxygalactonate 6-phosphate could be detected in either the cells or the growth medium. This strain did, however, produce the same amount of 2-oxo-3-deoxygalactonate as strains CO100 and CO 106.

Those 2-oxo-3-deoxygalactonate-resistant secondary mutants that were still sensitive to inhibition by galactonate contained normal activities of galactonate dehydratase and ODGalO kinase. It seems likely that in these mutants resistance was due to a defect in 2-oxo-3-deoxygalactonate transport (see later).

When this secondary-mutant selection procedure was repeated with galactonate rather than 2-oxo-3-deoxygalactonate as inhibitory substance, a similar pattern of resistance was found but with 80% of the galactonate-resistant mutants also being 2-oxo-3-deoxygalactonate-resistant. Of those mutants that were resistant to galactonate but still sensitive to 2-oxo-3-deoxygalactonate some, as anticipated, lacked galactonate dehydratase. Others, however, such as strain CO113 had normal activities of galactonate dehydratase and ODGalO kinase suggesting that they may be defective in galactonate transport. When cells of strain CO106 were used in uptake experiments [1-^{14}C]-galactonate was incorporated at a rate of 0.02 μmol min^{-1} mg dry weight^{-1}. However, strain CO113 accumulated radioactivity from [1-^{14}C]galactonate at less than 0.5% of the rate shown by strain CO106.

Genetic Mapping of Loci for the Galactonate Catabolic Enzymes

In preliminary mapping experiments the galactonate lesions in strains CO100, CO101 and CO102 were introduced into the F$^-$ strain PA309 by conjugation. Streptomycin-resistant recombinants that had inherited the ability to grow in the absence of a particular amino acid were selected on appropriately supplemented glucose plates and it was found that approximately 9% of the Arg$^+$ recombinants were galactonate negative. The various F$^-$ galactonate-negative recombinants were then print-mated with a number of the Hfr strains by the rapid mapping technique of Low (1973). Two Hfr strains, KL228 (Low, 1973) and AB2297 (Kornberg and Smith, 1969) of opposing polarity of chromosome transfer most readily gave galactonate-positive recombinants, indicating that the galactonate genes were located between min 78 and min 83 on the *E. coli* K-12 linkage map (Bachmann et al., 1976).

To locate the genes more precisely strain JM452 (*pyr*E, *uhp*) was used as recipient in a transduction experiment with donor phage grown on strain CO101 (*dgoD*). PyrE$^+$ transductants were selected and approximately 12% (22/178) of these were found to be galactonate negative. The orientation of the galactonate marker with respect to *pyr*E was determined by analysis of the unselected third marker in the cross, *uhp*. Of the four possible classes of transductant in this three-factor cross (Table 3) the least frequent class was *uhp*$^-$ *dgo*D$^-$. On the assumption that transductants arising from a minimum of four crossovers will arise significantly less frequently than those arising from a minimum of two crossovers, the *uhp*$^-$ *dgo*D$^-$ class would arise by four crossovers if the gene order was *pyr*E *uhp dgo*D. The high cotransduction frequency between *uhp* and *dgo*D seen in this cross was confirmed directly in an experiment with strain CO504 (*uhp*, *dgo*D) as recipient and donor phage grown on the wild-type strain K10. When galactonate-positive transductants were selected and analysed 61% (117/190) had also become Uhp$^+$. Using the equation of Wu (1966) to relate cotransduction frequency to map distance the 12% linkage to *pyr*E and the 61% linkage to *uhp*

suggested that *dgo*D was about 1 min from *pyr*E and 0.3 min from *uhp* which would place it near min 81.7 on the linkage map. Such a location suggested that *dgo*D should be highly co-transducible with the *dna*A locus which is located at min 81.7 on the linkage map (Bachmann et al., 1976). When this was tested the co-transduction frequency between *dgo*D and *dna*A was 85–90% and from a three factor cross the gene order was found to be *pyr*E *uhp dgo*D *dna*A (Orr, personal communication).

When the crosses were repeated using phage grown on strain CO100 (*dgo*A) a similar linkage to *pyr*E and to *uhp* was observed, indicating that the genes specifying galactonate dehydratase and ODGalOP aldolase are very closely linked. As already described no mutants lacking only ODGalO kinase have yet been obtained and the *dgo*K marker in the *dgo*A strain CO110 had to be located by a slightly different procedure. For this phage grown on strain CO110 (*dgo*A, *dgo*K) was used as donor with strain JM452 (*pyr*E, *uhp*) as recipient and PyrE$^+$ transductants selected. Those transductants that were also galactonate-negative were identified and when 24 of these galactonate-negative transductants were grown on glycerol they were found to produce galactonate dehydratase but were defective in both ODGalO kinase and ODGalOP aldolase, like the parental strain CO110. This close linkage between *dgo*A and *dgo*K was supported by the finding that strain CO110 could be transduced to galactonate-positivity with the same frequency as mutants defective only in ODGalOP aldolase.

The genetic location of the galactonate transport marker posed similar problems to the location of *dgo*K because it too was present in a strain additionally defective in ODGalOP aldolase. However, in this case the linkage between *dgo*T and *dgo*A could be measured directly by selection of 2-oxo-3-deoxygalactonate-positive transductants. Strain CO113 (*dgo*A, *dgo*T, *dgo*R, *odg*R) was the recipient and phage grown on strain CO103 (*dgo*R, *odg*R) was used to select the 2-oxo-3-deoxy-galactonate-positive transductants. Such transductants would need an active ODGalOP aldolase but would not require an active galactonate transport system for growth. When 98 were subsequently tested for growth on galactonate where the galactonate transport system would be required, all were found to be positive and must, therefore, have regained an active galactonate transport system. This suggested a very high co-transduction frequency between *dgo*A and *dgo*T.

Phage grown on the galactonate-negative mutants such as strains CO102 and CO117 that were defective in all of the galactonate catabolic enzymes were also used in crosses with strain JM452 as recipient and PyrE$^+$ transductants selected. Once again approximately 12% of the transductants had become galactonate negative and analysis of the *uhp* marker showed that the galactonate-negativity marker was located at min 81.7 on the linkage map.

Genetic Analysis of 2-Oxo-3-Deoxygalactonate-Positive Strains

The finding that the galactonate-negative transductants obtained from the cross between strain JM452 and strain CO110 produced galactonate dehydratase constitutively, suggested that a regulator gene (*dgo*R) controlling the formation of the galactonate catabolic enzymes in strain CO110 must also be located near min 81.7 on the linkage map. When phage grown on the galactonate constitutive strain CO103 was used to transduce the galactonate dehydratase-negative strain CO101 to galactonate positivity most surprisingly none of 130 transductants grew on 2-oxo-3-deoxygalactonate. They did, however, revert to give 2-oxo-3-deoxygalactonate-positive clones much more readily than did the wild-type strain K10. When 12 of the galactonate-positive 2-oxo-3-deoxygalactonate-negative transductants were grown on glycerol all were found to produce the galactonate enzymes constitutively. This suggested that the constitutive formation of the galactonate catabolic enzymes alone was insufficient to permit growth on 2-oxo-3-deoxygalactonate and that an additional mutation permitting 2-oxo-3-deoxygalactonate transport was necessary and must be present in the 2-oxo-3-deoxygalactonate-positive strain CO103. That this is the case was shown by an experiment in which strain CO106, the ODGalOP aldolase-negative derivative of strain CO103, was transduced to galactonate-positivity with phage grown on wild-type strain K10. This would be expected to introduce the normal form of the regulator gene (i.e. be inducible) but the mutation permitting 2-oxo-3-deoxygalactonate transport would still be present in the progeny since it was present in the recipient and is apparently unlinked to the *dgo* region. Such transductants were unable to grow on 2-oxo-3-deoxygalactonate and when 12 were grown on glycerol and cell-free extracts prepared, as expected none produced the galactonate enzymes constitutively. However, these transductants again readily reverted to growth on 2-oxo-3-deoxygalactonate and 5 such glycerol-grown 2-oxo-3-deoxygalactonate-positive revertants all now produced the galactonate catabolic enzymes constitutively. When strain CO109, the putative 2-oxo-3-deoxygalactonate transport-negative mutant derived from strain CO106 was similarly transduced to galactonate-positivity with phage grown on wild-type strain K10 the transductants were, as expect-

ed, unable to grow on 2-oxo-3-deoxygalactonate and in this case the transductants did not revert to growth on 2-oxo-3-deoxygalactonate.

Discussion

The absence of galactonate dehydratase, ODGalOP aldolase, and both of these enzymes plus ODGalO kinase from various mutants unable to grow on galactonate, strongly supports the earlier proposal (Deacon and Cooper, 1977) that these enzymes catalyse reactions that are necessary for galactonate catabolism in *Escherichia coli* K-12 (Fig. 1). Although no mutants lacking solely ODGalO kinase have yet been isolated the role of this enzyme in the formation of 2-oxo-3-deoxygalactonate 6-phosphate was demonstrated by strain CO110 that was defective in ODGalO kinase and ODGalOP aldolase and no longer accumulated 2-oxo-3-deoxygalactonate 6-phosphate during exposure to galactonate or 2-oxo-3-deoxygalactonate.

Growth inhibition by accumulated phosphorylated intermediates of sugar catabolism has been observed on many occasions (Fukasawa and Nikaido, 1961; Böck and Neidhardt, 1966; Beacham et al., 1968; Faik et al., 1971). It was thus not surprising to find that the accumulation of 2-oxo-3-deoxygalactonate 6-phosphate also caused growth inhibition. The site of inhibition must be a central biosynthetic process since growth inhibition in this case, as in others, is shown with either fermentative or oxidative growth substrates. The sensitive site(s) may not be the same for all phosphorylated compounds but it has been claimed recently that such inhibition caused by accumulated glycolytic intermediates is a dual effect due to an inhibition of cell-wall biosynthesis and an enhancement of catabolite repression (Irani and Maitra, 1977).

The close genetic location of galactonate permease, galactonate dehydratase, ODGalO kinase, ODGalOP aldolase, the regulator mutation that leads to constitutive production of these enzymes, and the putative promoter mutation that prevents their synthesis implies that the catabolic enzymes are likely to constitute an operon. The finding that in a mutant lacking galactonate dehydratase exposure to galactonate readily elicited the synthesis of ODGalO kinase and ODGalOP aldolase further suggests that galactonate itself rather than a catabolite derived from galactonate is the inducer of all the galactonate catabolic enzymes. The induction by galactonate was found to be subject to glucose catabolite repression.

In strain CO103 the galactonate catabolic enzymes are formed constitutively and the absence of all the catabolic enzymes in mutants such as strain CO117 (derived from strain CO103) cannot be due to a failure to induce the enzymes. The simplest explanation is that they are promoter mutants that fail to initiate transcription of the galactonate operon. Since the galactonate operon is close to the origin of chromosome replication in *E. coli* K-12 (Bachmann et al., 1976), rapidly growing cells which show multiple initiation of chromosome replication will contain several copies of the operon. This potential ready availability of the galactonate catabolic enzymes might indicate that galactonate is a common carbon and energy source for *E. coli*.

Although 2-oxo-3-deoxygalactonate and 2-oxo-3-deoxygluconate are chemically similar and wild-type *E. coli* K-12 is unable to grow on either compound without mutation, the nature of the changes necessary to permit growth on 2-oxo-3-deoxygalactonate are apparently different to those required to allow growth on 2-oxo-3-deoxygluconate (Lagarde et al., 1973). Whilst both compounds are catabolised through the action of specific kinases and aldolases to produce eventually glyceraldehyde 3-phosphate and pyruvate, it seems that 2-oxo-3-deoxygluconate can induce the enzymes necessary for its catabolism (Lagarde et al., 1973) whereas 2-oxo-3-deoxygalactonate apparently cannot. Thus only a single mutation leading to the constitutive expression of the 2-oxo-3-deoxygluconate uptake system will permit growth on 2-oxo-3-deoxygluconate, whereas two distinct mutations, one leading to the constitutive expression of the galactonate catabolic enzymes, the other permitting transport of 2-oxo-3-deoxygalactonate are both necessary before growth on 2-oxo-3-deoxygalactonate can occur. Although no extensive biochemical study has been made of the mutation that permits 2-oxo-3-deoxygalactonate transport the genetic experiments show that it is not closely linked to the galactonate operon. It remains to be seen whether it is the 2-oxo-3-deoxygluconate transport system that is active in transporting 2-oxo-3-deoxygalactonate.

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