

## Two Genes Affecting Glucarate Utilization in *Escherichia coli* K12

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D-Glucarate is transported into *Escherichia coli* K12 by an inducible system at an apparent rate of 7 to 15 nmol min<sup>-1</sup> (mg dry mass)<sup>-1</sup>. The apparent  $K_m$  for uptake is 16  $\mu$ M. The system is induced by growth on glucarate or glycollate. Galactarate competes with glucarate for the uptake system. A mutation (*garA*) was isolated in which activities of glucarate transport and glucarate dehydratase and the ability to grow on glucarate or galactarate are all impaired. The mutation maps at min 16. Another mutation of indistinguishable phenotype is probably a deletion of the genes *garB* and *tonA* at min 3.5.

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### INTRODUCTION

*Escherichia coli* K12 metabolizes D-glucarate (the 1,6-dicarboxylic acid derivative of glucose) by a pathway involving the enzymes D-glucarate dehydratase (EC 4.2.1.40), 2-keto-3-deoxy-D-glucarate aldolase (EC 4.1.2.20) and tartronate-semialdehyde reductase (EC 1.1.1.60) (Blumenthal & Fish, 1963). This paper examines the properties of the glucarate transport system and of mutants in which this transport system is deficient.

### METHODS

**Organisms.** The strains of *E. coli* used and their provenance are listed in Table 1. The genetic symbols are those of Bachmann *et al.* (1976), except *garA* and *garB*. Bacteriophage P1kc was obtained from M. D. Yudkin and T5 from P. Oliver.

**Growth.** Cultures were grown aerobically at 37 °C either in double-strength nutrient broth, or in defined medium containing salts (Ashworth & Kornberg, 1966), an energy source equivalent in carbon to 10 mM hexose, and L-amino acids (40 to 80  $\mu$ g ml<sup>-1</sup>) as required. Growth was monitored by diluting samples and measuring their absorbance at 680 nm in a 1 cm light path spectrophotometer cuvette. An  $A_{680}$  of 1.0 was taken as equivalent to 0.7 mg dry mass cells ml<sup>-1</sup>. The genetical methods employed were those described by Miller (1972).

**Measurement of [<sup>14</sup>C]glucarate transport.** Bacteria were harvested from growing cultures by centrifuging at 2000 *g* for 7 min, washed in 50 mM-sodium phosphate buffer pH 7.2 at room temperature, and suspended in 2 ml buffer containing chloramphenicol (100  $\mu$ g ml<sup>-1</sup>). Washed bacteria (0.7 mg ml<sup>-1</sup> final concentration unless otherwise stated) and buffer containing chloramphenicol were shaken for 3 min at 30 °C. [<sup>14</sup>C]Glucarate (91 nmol) was added (giving 1 ml final volume) and 0.1 or 0.2 ml samples were removed at appropriate times between 10 and 70 s. The samples were filtered, the cells were washed and the incorporated radioactivity was measured as described by Bächli & Kornberg (1975).

**Enzyme measurements.** Cell-free extracts were prepared from cells by sonication for 1 to 2 min (with intervals for cooling) in 50 mM-Tris/HCl buffer pH 7.5, followed by centrifugation at 27000 *g* for 15 min at 0 °C.

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Table 1. *Strains of Escherichia coli* used

Strain	Genotype	Reference or source
<b>Hfr strains</b>		
K1.1.4	<i>met thy gltA pps</i> (PO1)	Laboratory stock
KL16.21	<i>ptsM ptsF</i> (PO45)	Ferenci & Kornberg (1974)
KL208	<i>gar<sup>+</sup></i> (PO43)	Low (1973)
PB1139	<i>gar<sup>+</sup> galT</i> (PO1)	P. L. Bergquist
PB99	<i>gar<sup>+</sup> met</i> (PO43)	P. L. Bergquist
CSH68	<i>gar<sup>+</sup> met mtl mal</i> (PO58)	P. L. Bergquist
Hfr Cavalli	<i>gar<sup>+</sup> met</i> (PO2A)	Bachmann (1972)
W1895	<i>gar<sup>+</sup> met</i> (PO2A)	Bachmann (1972)
CS101	<i>garB met tonA</i> (PO2A)	Bachmann (1972)
K10	<i>garB tonA</i> (PO2A)	Bachmann (1972)
P4X	<i>garB<sup>+</sup> met</i> (PO3)	Bachmann (1972)
JM1343	<i>garB tonA mel ilv</i> (PO2A)	Mel <sup>-</sup> from JM1090 (Henderson <i>et al.</i> , 1977)
JM1447	<i>garB tonA mel ilv ara</i> (PO2A)	Ara <sup>-</sup> from JM1343
JM1448	<i>garB tonA mel ilv thr leu</i> (PO2A)	P1. K2.1t × JM1447
AT997	<i>gar<sup>+</sup> dapC</i> (PO45)	Bukhari & Taylor (1971)
JM1056	<i>gar<sup>+</sup> dapD</i> (PO45)	AT982 (Bukhari & Taylor, 1971)
<b>F<sup>+</sup> strain</b>		
W6	<i>gar<sup>+</sup> met</i>	Bachmann (1972)
<b>F<sup>-</sup> strains</b>		
K2.1.2.22	<i>gar<sup>+</sup> met his thr leu ppc pps ptsG str</i>	Laboratory stock
K2.1t	<i>gar<sup>+</sup> his arg thr leu xyl pps galP mglP str</i>	Brice & Kornberg (1967)
AR109	<i>garA met his thr leu ppc pps ptsG str</i>	K2.1.2.22 by resistance to glucarate on acetate
AR114	<i>garA his arg thr leu pps ptsG str</i>	AR109 by transduction
AR115	<i>gar<sup>+</sup> his arg thr leu pps ptsG str</i>	K2.1.2.22 by transduction
AR116	<i>garA arg thr leu pps ptsG ptsF ptsM str</i>	KL16.21 × AR114
AR127	<i>garA arg galT ptsG str</i>	PB1139 × AR116
PB68	<i>gar<sup>+</sup> pro leu lacY thi mtl xyl galK2 ara str</i>	P. L. Bergquist
AB1157	<i>gar<sup>+</sup> pro thr leu arg his lac str</i>	B. M. Wilkins

Glucarate dehydratase was measured essentially by the method of Blumenthal (1966) using a 10 min incubation at 30 °C. The assay mixture (final volume 2 ml) contained 20  $\mu$ mol potassium glucarate, 160  $\mu$ mol Tris/HCl buffer pH 8.0, 20  $\mu$ mol MgSO<sub>4</sub> and cell-free extract containing about 5 mg protein. Protein was determined by the Lowry method.

**Preparation of [<sup>14</sup>C]glucarate.** The oxidation of glucose to glucaric acid was based on the method described by Mehlretter (1963), suitably scaled down. [U-<sup>14</sup>C]Glucose [25 mg, 21  $\mu$ Ci mg<sup>-1</sup> (0.78 MBq mg<sup>-1</sup>); The Radiochemical Centre, Amersham] was heated at 75 °C for 2 h with 0.02 ml sodium nitrate (0.25 mg) and 0.32 ml conc. nitric acid. The excess nitric acid was removed by vacuum, and the crude glucaric acid was dissolved in water. One quarter of this was applied in a strip to Whatman 3MM chromatography paper and run in a high-voltage electrophoresis apparatus (5 kV, pH 6.5, 12 min). Three major bands were found by autoradiography, and the one corresponding to glucaric acid was eluted with water and reapplied to chromatography paper. This was again subjected to electrophoresis (5 kV, pH 3.5, 25 min), and one major and seven minor bands were found. The major band, glucaric acid, was eluted in 4.5 ml water and contained  $1.04 \times 10^7$  d.p.m. ml<sup>-1</sup>.

## RESULTS

**Isolation of a *garA* mutant.** Organisms deficient in the pathway of glucarate metabolism were isolated by the procedure of Kornberg & Smith (1969). A suspension of strain K2.1.-2.22 (*ppc*) grown in nutrient broth was spread on a plate containing 10 mM-sodium acetate and 30 mM-potassium glucarate as carbon sources. Glucarate, as well as other substrates catabolized to C<sub>3</sub>-compounds, inhibits the growth of Ppc<sup>-</sup> mutants on acetate. Mutants arising were presumably either Ppc<sup>+</sup> or had lost a component of the multistage pathway whereby glucarate entered the cells and gave rise to the C<sub>3</sub>-inhibitor of the glyoxylate cycle.

Table 2. *Specific activities of glucarate dehydratase in gar<sup>+</sup> and garA strains of E. coli*

Strains AR115 (*gar<sup>+</sup>*) and AR114 (*garA*) were grown in either nutrient broth, nutrient broth containing 10 mM-glucarate, or minimal medium containing amino acid growth requirements plus 30 mM-sodium glycollate. Glucarate dehydratase was assayed in the cell-free extracts; specific activities are expressed as nmol min<sup>-1</sup> (mg protein)<sup>-1</sup>.

Strain	Growth conditions	Glucarate dehydratase activity
AR115	Nutrient broth	19
AR115	Nutrient broth + glucarate	900
AR114	Nutrient broth + glucarate	28
AR115	Glycollate	370
AR114	Glycollate	4

Such mutants were tested for their ability to grow on acetate and acetate plus glucarate and for their inability to grow on other carbohydrates with or without acetate. One of these, AR109, and the parent strain K2.1.2.22 were transduced using bacteriophage P1kc grown on strain K2.1t as donor, and Ppc<sup>+</sup>Met<sup>+</sup> colonies were selected on medium containing fructose, histidine, arginine, threonine and leucine to obtain strains AR114 (*garA ppc<sup>+</sup>*) and AR115 (*gar<sup>+</sup> ppc<sup>+</sup>*). The *garA* mutation is unstable, and revertants appear with high frequency on plates containing glucarate as carbon source. Strain AR114 is also unable to utilize galactarate as carbon source. Glucarate-positive strains grown on galactarate or glycollate adapt to growth on glucarate without a discernible lag.

**[<sup>14</sup>C]Glucarate transport.** [<sup>14</sup>C]Glucarate was taken up rapidly by strain AR115 (*gar<sup>+</sup>*) grown in nutrient broth containing glucarate. The apparent rate of uptake by cells in saturating external concentrations of glucarate was about 7 nmol min<sup>-1</sup> (mg dry mass)<sup>-1</sup> at 37 °C and was linear for 4 min. Half the maximum velocity of glucarate transport was obtained with 16 µM-glucarate. The uptake of glucarate by cells was completely inhibited by 2 min preincubation with the uncoupler carbonyl cyanide *m*-chlorophenylhydrazone, at 25 µM final concentration. The *garA* strain AR114 took up [<sup>14</sup>C]glucarate very slowly (less than 10% of the control rate) and little accumulated. This slow rate may be an overestimate of uptake by *garA* cells because of selection of Gar<sup>+</sup> revertants during growth in the presence of glucarate (see Table 3). Strains with the *garB* lesion did not take up glucarate.

**Inhibition of glucarate uptake.** Chemically related compounds were tested for their ability to inhibit glucarate transport, using a 75-fold excess of potential inhibitor. Of the compounds examined, only galactarate (98% inhibition) was extremely inhibitory. Gluconate (4%), glucuronate (9%), diaminopimelate (7%), glutarate (17%) and cystine (29%) inhibited the rate of glucarate uptake by the amounts indicated.

**Glucarate dehydratase.** Glucarate dehydratase activity was measured in cell-free extracts of AR115 (*gar<sup>+</sup>*) and AR114 (*garA*) (Table 2). When the strains were grown in nutrient broth, the activities were very low. Addition of glucarate to the nutrient broth gave high activities for strain AR115, though not for strain AR114. Growth in minimal medium containing glycollate also gave medium activities with strain AR115, but not with strain AR114. No activity was detected in *garB* strains grown in nutrient broth in the presence of glucarate.

**Induction of glucarate transport and metabolism by glycollate.** Since glycollate and glucarate are both metabolized via tartronate semialdehyde, we investigated whether growth on glycollate would induce the enzymes necessary for growth on glucarate. All the strains related to Hfr Cavalli were unable to grow with glycollate as carbon source, but strains AR115 (*gar<sup>+</sup>*) and AR114 (*garA*) were both able to do so. Strains AR115 and AR114 grown on glycollate were incubated with glucarate plus glycollate, glycollate and glucarate; the cells were then harvested and assayed. The *gar<sup>+</sup>* cells took up glucarate at similar rates [approx. 15 nmol min<sup>-1</sup> (mg dry mass)<sup>-1</sup>], but the *garA* cells showed no transport activity (Table 3).

Table 3. *Uptake of [<sup>14</sup>C]glucarate by gar<sup>+</sup> and garA strains of E. coli*

Strains AR115 (*gar<sup>+</sup>*) and AR114 (*garA*) were grown overnight on sodium glycollate as carbon source and then washed and suspended at an  $A_{680}$  of 0.11 to 0.13 in 15 ml medium containing salts, required amino acids, potassium glucarate (6 mM) and/or sodium glycollate (20 mM) as indicated below. After incubation at 37 °C for 4 or 7 h, cells were washed and [<sup>14</sup>C]glucarate transport was measured.

Strain	Preincubation		$A_{680}$ after preincubation	Rate of glucarate transport [nmol (mg dry mass) <sup>-1</sup> min <sup>-1</sup> ]
	Addition(s)	Duration (h)		
AR115	Glucarate			
	+ glycollate	4	0.46	13
AR115	Glycollate	7	0.26	15
AR115	Glucarate	4	0.51	13
AR114	Glucarate			
	+ glycollate	7	0.23	<0.1
AR114	Glycollate	7	0.25	<0.1

*Mapping the garA locus.* Strain KL16.21 was mated with strain AR114 and a His<sup>+</sup> recombinant (strain AR116) was obtained that was *ptsG ptsM ptsF garA Gal<sup>+</sup>*. Interrupted mating experiments with strain KL208 as donor, selecting PtsG<sup>+</sup> recombinants, showed that the *garA* gene was located near *gal*. Strain AR127 (*garA Gal<sup>-</sup>*) was constructed by mating strain AR116 with strain PB1139, selecting for Pps<sup>+</sup>. Matings of this strain with the Hfr strains CSH68 and PB99 showed 79 to 97 % linkage between *garA* and *gal*.

Bacteriophage P1 grown on strain AR116 was used to transduce strain PB68, selecting GalK<sup>+</sup> recombinants. The yield of recombinants was very low, but of the 32 obtained, 11 were *garA*. The reason for the low yield of Gal<sup>+</sup> transductants is clear from the results of a transduction experiment in which bacteriophage P1 grown on strain AR114 was used to transduce strain K1.1.4 (*gltA*) to Glt<sup>+</sup>. Of 240 transductants examined, 67 were Gal<sup>-</sup>Gar<sup>-</sup>, 107 Gal<sup>-</sup>Gar<sup>+</sup>, 65 Gal<sup>+</sup>Gar<sup>+</sup> and 1 was Gal<sup>+</sup>Gar<sup>-</sup>. The most plausible explanation of this result is that the order of the genes is *...lac...gltA gal garA...trp...* and that strain AR114 is genotypically *gal* but carries an extragenic suppressor of the lesion. Since strain AR114 is ultimately derived from strain P678 it is reasonable that it should still carry components of the complex *gal<sub>6</sub>* (*gal-6*) mutation (Morse *et al.*, 1956; E. A. Adelberg, cited in Bachmann, 1972) and thus be genotypically *gal* but phenotypically Gal<sup>+</sup>.

The *garA* gene is not located between *gal* and *bio* since the *galbio* deletion strain JM759 (Jones-Mortimer & Kornberg, 1976), which is *garB*, may be transduced to Gar<sup>+</sup> (using bacteriophage P1 grown on strain P4X) without becoming Gal<sup>+</sup>Bio<sup>+</sup>.

*Mapping the garB locus.* While attempting to map the *garA* locus we observed that Hfr Cavalli strains were glucarate-negative. Analysis of recombinants from a cross between strain K2.1t (*thr leu*) and strain JM1343 (*mel garB*) suggested the gene order *mel thr leu garB*. This was confirmed by a cross between strain AB1157 (*thr leu gar<sup>+</sup>tonA<sup>+</sup>pro*) and strain JM1447 (*mel ara garB tonA*). The results of this cross (Table 4) indicate that *garB* and *tonA* are closely linked. Bacteriophage P1 was propagated on strains AT997 (*dapC*) and JM1506 (*dapD*). Glucarate-positive transductants of a *garB* strain were selected, and 120 from each cross were scored for their diaminopimelate requirement. The *dapD* gene was 96 % cotransducible with *garB*; the *dapC* gene was 87 % cotransducible. When P1 grown on strain P4X (wild type) was used to transduce strain JM1448, all of the 80 glucarate-positive strains examined were *tonA<sup>+</sup>* as judged by their sensitivity to bacteriophage T5. The *garB* lesion thus maps at min 3.5.

*Origin of the garB mutation.* All the strains shown in Chart 5 of Bachmann (1972) from W6 to K10 and miscellaneous related strains were scored for their ability to utilize glucarate as carbon source and for their sensitivity to bacteriophage T5. Strains W6, Hfr Cavalli,

Table 4. Analysis of Pro<sup>+</sup> recombinants from a cross between strain JM1447 (Hfr *garB tonA*) and strain AB1157 (F<sup>-</sup> *pro thr leu*)

Genotype				No.	Class
<i>thr</i>	<i>leu</i>	<i>garB</i>	<i>tonA</i>		
+	+	-	-	30	Donor
-	-	+	+	5	Recipient
-	-	-	-	5	
-	+	-	-	2	

*garB* and *tonA* are closely linked, and the gene order is *thr leu (garB, tonA) proA*.

W1895 and P4X were glucarate-positive and T5-sensitive; strains CS101 and K10 were glucarate-negative and T5-resistant.

# DISCUSSION

A substrate translocation mechanism exists for glucarate transport in *E. coli*; it is sensitive to uncouplers of oxidative phosphorylation and is half-saturated by external glucarate concentrations of approximately 16  $\mu\text{M}$ . The glucarate transport system is induced by glucarate, as is the enzyme glucarate dehydratase. It does not seem that internal glucarate is necessary for induction of the system, since glycollate also induces these functions.

Cells grown on galactarate are able to grow on glucarate without a lag, and vice versa. Glucarate dehydratase and galactarate dehydratase are both present when cells are grown either on glucarate or galactarate (Blumenthal, 1966; Blumenthal & Jepson, 1966). These acidic carbohydrates may share a transport system, particularly as excess galactarate almost completely inhibited glucarate uptake in glucarate-grown cells. The glucarate transport system is not used extensively by either of the closely related monocarboxylic acid carbohydrates gluconate and glucuronate, judging from competition experiments.

One of the genes for glucarate utilization, termed *garA*, has been located at about min 16 on the *E. coli* linkage map, and is cotransducible with *gal*. It appears that mutation of this gene is pleiotropic, since the transport of glucarate and glucarate dehydratase have much lower activities in both glucarate- and glycollate-induced *garA* cells; and the mutation also prevents growth on galactarate. Another gene, termed *garB*, has the same phenotype, but is located at about min 3.5. The *garB* lesion examined is probably a deletion of a gene (or genes) involved in glucarate catabolism and the gene for the T1,T5 receptor: it is stable, it cannot be separated by recombination from *tonA* and it appears to have arisen at the same time. Several hypotheses would account for the properties of these mutations: we have no evidence to distinguish between them.

The teleological question might be asked why *E. coli* possesses a catabolic pathway for glucarate. Perhaps the answer lies in the fact that normal humans excrete 4 to 30  $\mu\text{mol}$  D-glucarate  $\text{d}^{-1}$  in urine, and this is increased to values up to 300  $\mu\text{mol}$   $\text{d}^{-1}$  in people taking a variety of drugs including anticonvulsants (Hunter *et al.*, 1971). Such increased excretion reflects stimulation of the hepatic pathway of glucuronate metabolism (Aarts, 1971). As an occasional unwelcome coloniser of the bladder, *E. coli* might have evolved the ability to catabolize glucarate, particularly as physiological concentrations are similar to the  $K_m$  of glucarate transport. If so, the glucose content of urine (250  $\text{mg}$   $\text{d}^{-1}$ ) would probably repress glucarate catabolizing enzymes at first, until bacterial numbers increase to the extent where glucose is used up and less favoured energy sources are in demand.

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