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Mutations Affecting Gluconate Metabolism in *Escherichia coli*

ROSA NAGEL DE ZWAIG, NOÉ ZWAIG,[1] TOMÁS ISTÚRIZ,[2] AND REINA S. SÁNCHEZ

Departamento de Biologıa, Celular, Facultad de Ciencias, Universidad Central de Venezuela, Apartado 10098, Caracas, Venezuela

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A mutant of *Escherichia coli* K-12 that does not ferment gluconate on fermentation plates was isolated and characterized. This mutant, designated M2, shows a long lag for growth on gluconate mineral medium and somewhat reduced levels of high-affinity transport, gluconokinase, and gluconate-6-P dehydrase activities in the log phase of growth. The mutation involved is near *malA*. Deletion mutants in which *malA* region was affected were also studied. They were found to affect the function of different genes involved in gluconate metabolism.

After entering the cell, gluconate is phosphorylated to gluconate-6-P by the action of an inducible kinase (2; EC 2.7.1.12). Evidence has been presented which shows the operation in *Escherichia coli* of two gluconokinases (Hung et al., Bacteriol. Proc., p. 146, 1970). Gluconate-6-P may either be oxidized to pentose-P or metabolized through the Entner-Doudoroff (4) pathway (3, 8). The enzymes involved in this pathway are: gluconate-6-P dehydrase (EC 4.2.1.12), which is also inducible by gluconate, and the partially constitutive (3, 8) 2-keto-3-deoxygluconate-6-P aldolase (EC 4.2.1.14; see Fig. 1). Mutations affecting these two enzyme activities have been identified and described (5, 6, 14, 15, 18). Both mutations were found to lie at about 3 min from *his* (5, 6).

This paper describes the biochemical and genetic characteristics of a new mutation that impairs gluconate metabolism, as well as the behavior of strains carrying deletions affecting the utilization of this carbohydrate. A preliminary report of this work has been presented (Nagel de Zwaig et al., Abstr. Annu. Meet. Amer. Soc. Microbiol. 1972, p. 160).

MATERIALS AND METHODS

Media. The following media were used: LB (tryptone-yeast extract) broth and agar (12); mineral medium [3.4×10^{-2} M NaH_2PO_4, 0.064 M K_2HPO_4, 0.02 M $(NH_4)_2SO_4$, 10^{-6} M $FeSO_4$, 3×10^{-4} M $MgSO_4$, 10^{-6} M $ZnCl_2$; titrated with HCl to final pH 7.0]. Glucose or gluconate was added at 0.2%, vitamin B_1 at 1 μg/ml, L-amino acids at 20 μg/ml, and streptomycin at a final concentration of 200 μg/ml. EMB-agar (13); gluconate tetrazolium indicator plates (TTZ-gluconate) according to Signer et al. (17); casein hydrolysate (mineral medium supplemented with 1% casein hydrolysate).

Bacterial strains. Bacterial strains used are listed in Table 1.

P1 transduction. P1 lysates were prepared by the method of Lennox (11). For P1 transduction, the procedure of Rothman (16) was followed.

Mating procedures. Matings were carried out by standard procedures (10).

Mutagenesis. For mutagenesis, the procedure of Adelberg et al. (1) was used.

Preparation of cell extracts. Cell extracts were prepared from cultures harvested in the presence of 50 μg of chloramphenicol per ml while in the logarithmic phase of growth. The washed pellets were suspended in cold 0.1 M potassium phosphate buffer (pH 7.0) and disrupted in an MSE ultrasonic disintegrator (Measuring & Scientific Equipment, Ltd., London, England). The extract was centrifuged at $35.000 \times g$ for 10 min at 2 C. Protein was determined by the biuret procedure (19).

Growth of bacteria. Bacteria were grown at 37 C, in volumes of 20 ml, in flasks (250 ml) fitted with side arms, on a gyratory shaker (New Brunswick), at about 200 cycles/min. Growth was monitored by reading the turbidity in a Klett colorimeter with a no. 42 filter (one Klett unit corresponds to 4×10^6 cells/ml).

Enzyme assays. Gluconokinase and gluconate-6-P dehydrase were assayed by the procedure of Fraenkel and Horecker (7).

Assay of [^{14}C]gluconate uptake. Washed cells were suspended in 0.1 M tris(hydroxymethyl)-aminomethane (Tris)-hydrochloride buffer (pH 7.5) at a concentration of 5×10^7 cells/ml, and incubated with [^{14}C]gluconate in the presence of chloramphenicol (50 μg/ml) for 1 min. A sample of

[1] Present address: Departmento de Biologia, Fundoción Bariloche, Casilla de Correo 138, Bariloche, Argentina.

[2] Present address: Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Mass. 02115.

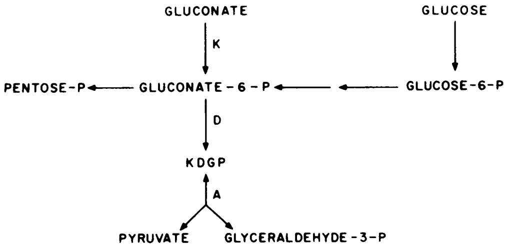

FIG. 1. *Metabolism of gluconate in E. coli. Abbreviations: KDGP, 2-keto-3-deoxygluconate-6-P; K, gluconokinase; D, gluconate-6-P dehydrase; A, KDGP aldolase.*

TABLE 1. *Bacterial strains*

Strain	Relevant characteristics	Origin
Escherichia coli K-12 derivatives		
M1	HfrC prototrophic	C. Levinthal (originally E15 through E. Lin
M2	HfrC prototrophic *gnt*	Derived from M1
MT18	HfrH *asd malA*	M. Schwartz
JC411	F⁻ *argG leu metB malA xyl mtl str*	D. J. Clark through D. G. Fraenkel
HfrG6	Hfr *his*	M. Schwartz
PA505MAΔ101	*mal arg met*	M. Schwartz
PA505MAΔ108	*bio mal arg met*	M. Schwartz

[a] Symbols: *gnt*, gluconate; *asd*, diaminopimelic acid plus homoserine; *mal*, maltose; *arg*, arginine; *leu*, leucine; *met*, methionine; *xyl*, xylose; *mtl*, mannitol; *str*, streptomycin; *his*, histidine; *bio*, biotin; *glpR*, glycerol phosphate regulator; *glpD*, glycerol phosphate dehydrogenase; *glg*, glycogen; Δ, deletion. The characteristics of strains carrying deletions are explained in Fig. 3 and Table 5.

this suspension was withdrawn, and the cells were collected on a membrane filter (0.45-μm pore size; Millipore Corp., Bedford, Mass.) and washed with 10 ml of the same buffer with suction. The filter was dried in scintillation vials to which 2 ml of scintillation fluid [4 g of 2,5-diphenyloxazole and 0.05 g of 1,4-*bis*-2-(5-phenyloxazolyl)-benzene per liter of toluene] was added. The radioactivity was counted in an automatic scintillation spectrometer (model 720, Nuclear-Chicago Corp.).

Chemicals. [U-^{14}C]gluconate (specific radioactivity, 4.1 Ci/mole) was obtained from The Radiochemical Centre (Amersham/Searle Corp.). D-Gluconic acid (potassium salt), 6-phosphogluconic acid (trisodium salt), pyruvate kinase, and lactic dehydrogenase were purchased from Sigma Chemical Co. Culture media were purchased from Difco Laboratories, Detroit, Mich. All the other chemicals employed were of the highest purity commercially available.

RESULTS

Selection of mutants affecting gluconate metabolism. Mutants of strain M1 in which gluconate metabolism was affected were selected as white colonies on EMB-gluconate plates after nitrosoguanidine treatment. Two types of mutants were obtained: a representative of one of these classes, designated M24, was characterized as negative for gluconate-6-P dehydrase; the mutation was mapped by mating near gene *his*. Mutant M24 is similar to the *edd* mutant studied by Fraenkel and collaborators (14, 18). The other mutant, designated M2, showed a 5-h lag when transferred from casein hydrolysate to gluconate mineral medium (Fig. 2). This strain, which also appears as negative on TTZ-gluconate plates, was studied further.

After the lag period on gluconate, strain M2 starts to grow with a generation time similar to that of strain M1 (Fig. 2). Cells of strain M2 growing at maximal rate on gluconate mineral medium were collected and analyzed for transport, gluconokinase, and gluconate-6-P dehydrase activities. All of these activities are present in strain M2, but at somewhat lower levels than the corresponding activities in the parental strain M1 grown under identical conditions (Table 2). Strain M2 gives very poor induction of the gluconate system when grown on casein hydrolysate plus gluconate. Since we cannot

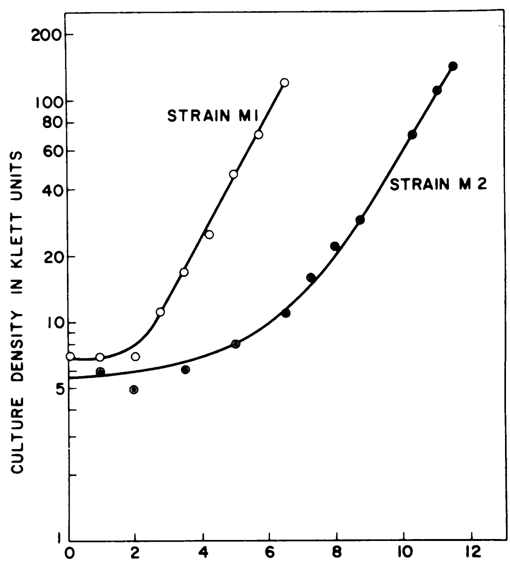

FIG. 2. *Growth of wild type and strain M2. Cells were pregrown in casein hydrolysate up to 200 Klett units (log phase). Growth was followed after dilution in mineral medium plus gluconate.*

TABLE 2. *Induction of the components of the gluconate system*[a]

Strain	Carbon source	Gluconate transport (counts/min)	Gluconokinase	Gluconate-6-P dehydrase
M1	Glucose	47	6.1	<1
	Gluconate	845	137	68
M2	Glucose	32	5.2	<1
	Gluconate	543	100	35

[a] Cells were collected at 100 Klett units, after growth on mineral medium plus the carbon source at a concentration of 0.2%. Specific activities of enzymes are expressed in nanomoles per minute per milligram of protein. The concentration of ^{14}C-gluconate was 2×10^{-5} M. This concentration is the calculated K_m of the transport system (Nagel de Zwaig et al., Abstr. Annu. Meet. Amer. Soc. Microbiol, 1972, p. 160; and unpublished data).

attribute the gluconate-negative character of strain M2 to a definite loss of any of the activities involved in gluconate metabolism, we will designate this mutation as *gntM2*.

The mutation of strain M2 seems to affect specifically gluconate metabolism since this strain ferments glucose, glycerol, xylose, lactose, glucuronate, galactose, arabinose, maltose, and mannitol on EMB plates.

Mapping of mutation gntM2. In order to establish the genetic location of *gntM2*, a mating experiment was performed with strain M2 as donor and strain JC411 as recipient. Different genetic recombinants were selected and purified, and their gluconate-fermenting ability was determined by streaking on EMB-gluconate plates. The results of this experiment (Table 3) revealed high linkage between *gntM2* and *malA*.

To determine more precisely the location of *gntM2*, a lysate of phage P1 grown on strain M2 was used to transduce strain MT18 (*gnt*$^+$ *mal asd*). Transductants *mal*$^+$ and *asd*$^+$ were selected and tested for their *gnt*$^+$ or *gnt* phenotype (Table 4). In all these transduction experiments, about 60% of the *asd*$^+$ transductants are *malA*$^+$, whereas 100% of the transductants selected as *mal*$^+$ are *asd*$^+$. The cause of this anomaly is not yet understood. The fact that *gnt* appears among *asd*$^+$ transductants with a lower frequency (56%) than *malA*$^+$ (62.5%), and that all the *asd*$^+$ *gnt* transductants are *malA*$^+$, suggests that the order of these markers is: *gntM2 malA asd*. The frequency of *gnt* among *malA*$^+$ recombinants is 88% (Table 4). This result is in agreement with the order of markers indicated above; if *gntM2* were located between *malA* and *asd*, all or almost all of the *malA* recombinants would be expected to be *gnt*.

Analysis of deletions of the malA region. The proximity of the *gntM2* mutation led us to examine the behavior of strains carrying different deletions of the *malA* region. The approximate lengths of the deletions of the strains studied are indicated in Fig. 3. Most of these strains showed anomalies in gluconate utilization (Table 5). Deletion strains HfrG6ΔMD2, HfrG6ΔMD3, and HfrG6ΔMD18, like their parental strain HfrG6, did not show impairment in the fermentation of glucose, arabinose, xylose, lactose, ribose, mannose, or mannitol on EMB plates.

Strain HfrG6ΔMD18 grows very poorly on gluconate mineral medium, does not transport gluconate, and gives low levels of gluconokinase and no gluconate-6-P dehydrase activity. Pseudorevertants of this deletion strain, which show better growth on mineral gluconate medium, give high activities of gluconokinase and gluconate-6-P dehydrase (Table 6). This indicates that the genes for these activities have not been lost by the deletion. These pseudorevertants appear to involve a mutation in a regulatory gene since they show either

TABLE 3. *Mapping of gntM2 by mating between strains HfrC M2 (gnt) and F$^-$ JC411 (gnt$^+$)*[a]

Selected recombinants	Frequency (%)	Frequency (%) of a nonselected marker							
		leu$^+$	*met*$^+$	*mtl*$^+$	*xyl*$^+$	*malA*$^+$	*argG*$^+$	*his*$^+$	*gnt*
leu$^+$*str*	2		20	1.2	0	0	0	0	0
met$^+$*str*	1.4	22.8		0	0	0	0	0	0
xyl$^+$*str*	0.06	3.8	0	19		15.0	38	0	11.4
malA$^+$*str*	0.04	6.4	3.2	0	15.2		6.4	0	55.0
his$^+$*str*	0.01	13.2	3.8	1.2	0	0	10.8		1.2

[a] Eighty colonies of *leu*$^+$*str*, *met*$^+$*str*, or *his*$^+$*str* recombinants and 30 colonies of *xyl*$^+$*str* or *mal*$^+$*str* recombinants were analyzed for the acquisition of nonselected markers. Selection of recombinants receiving *leu*$^+$, *his*$^+$, or *met*$^+$ markers from the Hfr were selected on glucose-mineral-agar plates supplemented with the corresponding requirements and streptomycin. The *malA*$^+$ and *xyl*$^+$ recombinants were selected on EMB-maltose or EMB-xylose plates, respectively.

constitutive levels of gluconokinase, gluconate-6-P dehydrase and low-affinity transport, or high inducible levels of all these activities. None of the pseudorevertants studied recovered the capacity to transport gluconate at 2×10^{-5} M; however, they were all able to transport gluconate at 5×10^{-4} M.

The two strains carrying the largest deletions, HfrG6ΔMD2 and HfrG6ΔMD3, have similar phenotypes (Table 5). They do not ferment gluconate on EMB or TTZ plates, do not transport gluconate at concentrations of 2×10^{-5} M or 5×10^{-4} M, lack gluconokinase activity, and they both show constitutive levels of gluconate-6-P dehydrase. The study of the pseudorevertants of strain HfrG6ΔMD18 indicates that gluconokinase, gluconate-6-P dehydrase, and low-affinity transport are controlled by a common regulatory gene. Since strains HfrG6ΔMD2 and HfrG6ΔMD3 show derepressed levels of gluconate-6-P dehydrase, the lack of activity of gluconokinase and low-affinity transport are interpreted to be due to the loss of the structural genes for these activities.

Strain PA505MAΔ101 carries a deletion that extends from inside the *malA* locus to a point

TABLE 4. *Mapping of gntM2 by P1 transduction*[a]

Selected marker	Frequency (%) of nonselected markers			
	malA⁺	*asd*⁺	*gnt*	*malA*⁺*gnt*
asd⁺	62.5		56	56
malA⁺		100	88	

[a] Phage P1 was grown on strain M2; strain MT18 (*asd malA gnt*⁺) was used as recipient. Two hundred colonies of each type of transductant were analyzed for nonselected markers. The *asd*⁺ transductants were selected on glucose-mineral plates supplemented with thiamine.

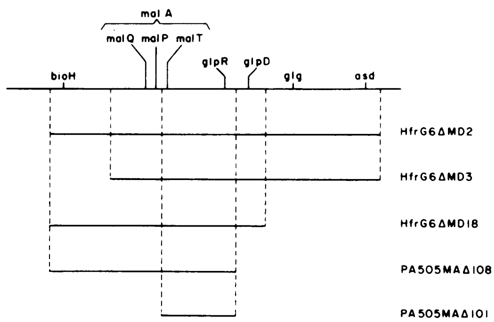

FIG. 3. *Approximate lengths of the deletions carried by strains of E. coli (9; M. Schwartz, personal communication). The characteristics of these strains are explained in the text and in Table 5.*

between *glpR* and *glpD* (9). Deletion of this region does not lead to any apparent anomaly in gluconate metabolism: normal levels of transport, gluconokinase, and gluconate-6-P dehydrase are observed (Table 5). This finding suggests that the deleted region does not contain genes involved in gluconate utilization.

As the chromosome is diagrammed in Fig. 3, the deletion of strain PA505MAΔ108 has the same right end limit as does strain PA505MAΔ101, but it extends to the left beyond *bioH*. This strain has a phenotype similar to that of strain HfrG6ΔMD18; it grows slowly in gluconate mineral medium after a very long lag; it does not transport gluconate at 2×10^{-5} M, and it gives poor induction of gluconokinase and gluconate-6-P dehydrase after growth on casein hydrolysate medium plus gluconate. A pseudorevertant derived from this deletion strain shows somewhat higher values of gluconokinase, gluconate-6-P dehydrase, and low-affinity transport, but does not transport gluconate at 2×10^{-5} M. We do

TABLE 5. *Relevant characteristics of the deletion carrying strains.*

Strain	Test on plates with gluconate			^{14}C-gluconate uptake (counts/min)[a]		Glucono-kinase	Gluconate-6-P dehydrase	Regulation
	EMB	TTZ	Mineral[b]	2×10^{-5} M	5×10^{-4} M			
HfrG6ΔMD2	−	−	−	−	−	−	+	c
HfrG6ΔMD3	−	−	−	−	−	−	+	c
HfrG6ΔMD18	−	+	−	−	−	+ −	−	i
PA505MAΔ108	−[c]	+	−	−	ND	+ −	+ −	i
PA505MAΔ101	+	+	+	+	ND	+	+	i

[a] ^{14}C-gluconate uptake was determined at gluconate concentrations of 2×10^{-5} M and 5×10^{-4} M; these are the approximate K_m values of the high- and low-affinity transport systems for gluconate, respectively (Nagel de Zwaig et al., Abstr. Annu. Meet. Amer. Soc. Microbiol. 1972, p. 160; and unpublished data).

[b] Growth was recorded after 24 h of incubation at 37 C.

[c] Appeared as + in some batches of EMB gluconate; c, constitutive; i, inducible; ND, not determined; + −, lower activity than that of the corresponding parental strain. The activities were measured after growth on casein hydrolysate with or without gluconate.

TABLE 6. *Pseudorevertants derived from strain HfrG6ΔMD18*

Strain[a]	Carbon source	^{14}C-gluconate uptake (counts/min)[b]		Gluconokinase	Gluconate-6-P dehydrase
		2×10^{-5} M	5×10^{-4} M		
HfrG6	Casein hydrolysate	42	501	25	<1
	Casein + gluconate	1,131	2,140	626	171
HfrG6ΔMD18	Casein hydrolysate + gluconate	25	430	176	<1
Rev. 1	Casein hydrolysate	48	690	53	<1
	Casein + gluconate	89	7,160	1,337	171
Rev. 2	Casein hydrolysate	87	7,450	1,034	288
	Casein + gluconate	53	4,340	1,251	179

[a] Cells were grown on 1% casein hydrolysate with or without 0.4% gluconate up to 200 Klett units. Rev. 1 and Rev. 2, were isolated as gluconate fermenters on EMB-gluconate plates.

[b] For conditions, see Materials and Methods and Table 5.

not know whether the differences observed between strains HfrG6ΔMD18 and PA505MAΔ108 (Table 5) are due to the different extension of the deletions involved or to differences in the host strains.

From the characteristics of the deletion strains studied, we can infer the following. (i) Genes affecting low-affinity transport of gluconate and gluconokinase activities, as well as a regulatory gene, lie near *asd*, in the region extending from *glpD* to the right end (as represented in Fig. 3) of deletions HfrG6ΔMD2 and HfrG6ΔMD3; this regulatory gene should exert negative regulation since its loss leads to constitutive gluconate-6-P dehydrase activity. (ii) A gene (or genes) affecting transport of gluconate at 2×10^{-5} M lies in a deleted region common to deletions PA505MAΔ108 and HfrG6ΔMD18; since deletion strain PA505MAΔ101 does transport gluconate at 2×10^{-5} M, the gene for this transport activity should be located in the region extending from *malA* to the left of *bioH*.

DISCUSSION

Mutant strain M2 shows a long lag for growth on gluconate mineral medium; in the log phase of growth, all known activities involved in gluconate metabolism are present, though at somewhat reduced levels. The *gntM2* mutation has been mapped by P1 transduction close to the locus *malA*, between genes *bioH* and *malA*. The function of this gene is not yet well understood; *gntM2* could be interpreted as a superrepressor type of mutation, but the fact that it lies in a different location from that of a regulatory gene of the system (20) and that it is recessive to the wild-type allele in the diploid state (Diéguez and Nagel de Zwaig, unpublished data) seems to exclude this possibility. This mutation is further discussed in the accompanying paper (20).

Characterization of different deletion strains of the *malA* region revealed that several genes involved in gluconate utilization are located in this region. Analysis of these strains indicates the following. (i) A deleted region common to strains HfrG6ΔMD18 and PA505MAΔ108, that does not include the *malA glpR* region, contains a gene (or genes) involved in the transport of gluconate at 2×10^{-5} M. (ii) A region that includes the *glg* and *asd* genes also contains: a gene (or genes) responsible for gluconate uptake at a concentration of 5×10^{-4} M, a negative regulatory gene, and a gluconokinase activity. In confirmation of the former conclusion, a point mutation affecting a gluconate low-affinity transport activity has been mapped close to gene *asd* by P1 transduction (Nagel de Zwaig et al., Abstr. Annu. Meet. Amer. Soc. Microbiol. 1972, p. 160; and unpublished data). Furthermore, mutants involving a regulatory gene for the gluconate system have also been isolated and mapped near gene *asd* (20). Finally, mutations affecting two different gluconokinase activities have been isolated; one of these mutations lies close to gene *asd* (Diéguez and Zwaig, unpublished data).

Genetic studies on the gluconate system have so far revealed that the genes involved are located in three different regions of the *E. coli* chromosome. The work of Fraenkel and collaborators (6, 14) and Faik et al. (5) showed that the genes responsible for gluconate-6-P dehydrase and 2-keto-3-deoxygluconate-6-P aldolase activities lie at a point represented at about 3 min from *his* on the chromosome map; the results reported in this paper reveal two other regions: one near *malA*, involved in the expression of a high affinity transport activity; another close to *asd*, carrying genes affecting regulation, low-affinity transport, and gluconokinase activities.

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