ISOLATION AND PROPERTIES OF *E. COLI* MUTANTS AFFECTED IN GLUCONATE UPTAKE

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1. Introduction

Enteric bacteria convert external gluconate to intermediates of central metabolic pathways by a process that involves at least four steps. Gluconate must first be taken up by the cells. There is evidence that this uptake is catalysed by an inducible process that, in vesicles prepared from *E. coli*, can be coupled to the oxidation of D-lactate [1]; it has also been briefly reported [2] that this process can be catalysed by two distinct transport systems that differ in their affinity for gluconate. Gluconate kinase (EC 2.7.1.12) catalyses the phosphorylation of the gluconate thus taken up with concomitant conversion of ATP to ADP [3]; it has been reported [2,4] that *E. coli* also contain two gluconate kinases. 6-Phosphogluconate thus formed can then be catabolized via pentose phosphates, and via the Entner–Doudoroff pathway [5] which has been shown [6] to be of predominant importance for gluconate dissimilation by *E. coli*.

Although the isolation and properties of mutants impaired in one or more component steps of 6-phosphogluconate catabolism have been described [7–9], mutants impaired in the uptake and phosphorylation of gluconate have been reported only in brief abstracts [2,4]. It is the main purpose of the present paper to remedy this deficiency. A simple procedure is described for the isolation, recognition and analysis of mutants that are impaired in one gluconate uptake system although they can be induced to form a second: we propose to designate the genetic marker specifying this missing uptake system for gluconate *usgA*. These mutants also form gluconate kinase to a somewhat lower specific activity than do wild-type cells: it is possible that the process used for their isolation favours the selection of polar mutants or of organisms carrying deletions of *usgA* and one of the two postulated gluconate kinases.

2. Experimental

The organisms used in this work are listed in table 1. The procedures used for their growth, for measurement of the rates of uptake of labelled substrates by washed cell suspensions, and for genetic analyses, were as previously described [10–12]. Methods for the isolation of mutants impaired in gluconate utilization are reported in the following section.

3. Results and discussion

3.1. *Isolation of mutants*

The addition of gluconate (or of other substances, such as glucuronate or galacturonate, that are also metabolically converted to phospho-2-keto-3-deoxygluconate) to mutants devoid of the aldolase that catalyses the cleavage of phospho-2-keto-3-deoxygluconate to pyruvate and glyceraldehyde 3-phosphate [13], leads to the accumulation of the phosphorylated ketoacid and consequent growth stasis [8, 9, 14]. For the same reason, such *kdgA* mutants do not grow on solid media if these contain gluconate as well as another, normally utilizable, carbon source. However, when such plates, of media containing 20 mM glycerol and 5 mM gluconate, were inoculated with ca. 10^8 cells of *kdgA*-mutant A314 (table 1)

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Table 1
Organisms used in this study.

Strain	Derivation or reference	Genetic markers*	Response to streptomycin	Mating type
P4X	[21]	*metB*	S	Hfr
A314	[21]	*metE, kdgA*	S	Hfr
PF1	A314	*metB, uxgA*	S	Hfr
A1201	PA309	*arg(EH), trp, xyl, malA, gal, kdgA*	R	F^-
Δ70	[PA309 × P10-*dct1*] [19, 20]	*metB, trp, his, arg(HCBE) thr, leu, malA*, Δ[*dct–kdgK*]	R	F^-

* Where possible, the abbreviations used are those listed by Taylor [18]. In addition, *uxg* indicates the inability to take up gluconate described in this paper; *kdgA* and *kdgK* specify, respectively, the structural genes for phospho-2-keto-3-deoxygluconate aldolase and 2-keto-3-deoxygluconate kinase; Δ[*dct–kdgK*] a deletion extending from the *dct* to the *kdgK* markers on the *E. coli* genome.

and were incubated at 30° or 37° for 2–3 days, approx. 10^2 colonies appeared that were now resistant to the presence of gluconate but were still devoid of phospho-2-keto-3-deoxygluconate aldolase activity, as shown by their inability to grow on hexuronates as sole carbon source. Indeed, these organisms resistant to the presence of gluconate were still sensitive to the presence of hexuronates: growth on 20 mM glycerol was arrested by the addition of 5 mM glucuronate or galacturonate although it was unaffected by the addition of 5 mM gluconate. The change undergone by strain A314 in acquiring this resistance to gluconate thus involved a mutation that prevented the formation of the (still toxic) phospho-2-keto-3-deoxygluconate from gluconate in the medium.

There are a number of ways in which the pathway from external gluconate to intracellular phospho-2-keto-3-deoxygluconate via 6-phosphogluconate, can be interrupted; mutants affected in one or more of these steps can be readily recognized by an adaptation of the technique first described by Zwaig and Lin [15] and subsequently used for the detection of mutants impaired in the uptake of methyl α-glucoside [16] or constitutive for the uptake of hexose phosphates [17]. In this procedure, mutants are screened for their ability to take up [^{14}C]gluconate during their growth on plates of nutrient agar that also contain the isotopic material in low concentration (≤ 10 μM). By the use of this technique, many colonies were obtained from strain A314 that did not take up [^{14}C]gluconate under these conditions. One of these was converted to $kdgA^+$ by phage P1-mediated transduction [8] and the resultant organism, designated PF1, was used for further study. (A culture of strain PF1 has been deposited with the *E. coli* Genetic Stock Center, Department of Microbiology, Yale University Medical School, New Haven, Conn., USA).

3.2. *Properties of the mutant PF1*

As expected from the method used for its recognition, the mutant PF1 was markedly impaired in its ability to take up [^{14}C]gluconate. When incubated with 0.1 mM sodium [U-^{14}C]gluconate at 30°, a washed suspension of the mutant, that had been grown for many generations on a medium containing 20 mM glycerol and 10 mM sodium gluconate, took up less than 10% of the isotope that was taken up by a similarly-treated suspension of the wild-type *E. coli* (fig. 1). Since this experimental procedure measures both the entry of labelled gluconate into the cells and its incorporation into stable cell components, it was possible that this impairment of uptake was associated with a dysfunction of transport, of gluconate kinase, or both.

The presence of a lesion in strain PF1 that affects more than one parameter of gluconate utilization is indicated by the growth properties of the mutant, and by the specific activities at which enzymes of

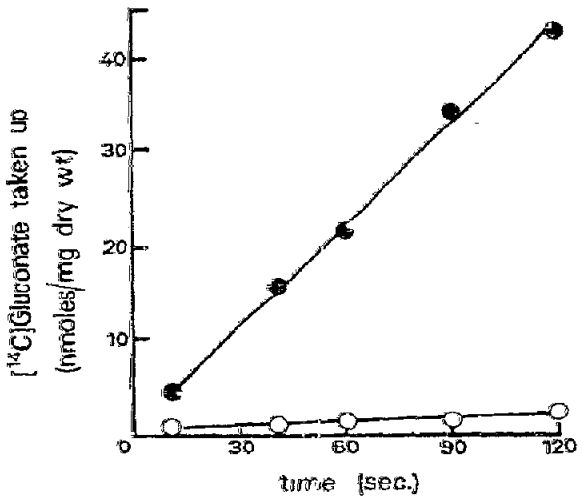

Fig. 1. Uptake of 0.1 mM sodium [U-^{14}C]gluconate by washed suspensions of *E. coli* strains P4X (•) and PF1 (○). The organisms had been grown overnight on media containing 20 mM glycerol plus 10 mM gluconate as carbon source.

gluconate catabolism are formed during growth. Although, like wild-type *E. coli*, the mutant PF1 grew readily on glucose, glycerol, glucuronate or on a variety of gluconeogenic substrates, cultures of strain PF1 required over 7 hr for one cell doubling to occur when the organism was transferred to aerobic growth at 37° with gluconate as sole carbon source. However, a progressive acceleration of growth rate was observed throughout this period, which continued until, after a day or more, the doubling time of the cells had approached that of wild-type *E. coli*. This was not the consequence of selecting a further mutant, or a revertant to wild-type, but was associated with the induction of a gluconate uptake system with properties different from those normally predominating in wild-type *E. coli* grown on gluconate (fig. 2). In contrast to such suspensions of wild-type cells, which readily take up [^{14}C]gluconate from solutions in the micromolar range (apparent $K_m = 1 \times 10^{-5}$ M), suspensions of gluconate-grown PF1 require much higher gluconate concentrations for uptake at significant rates, and the apparent K_m of this process is of the order of 0.1 mM.

The slow adaptation to an alternative and apparently more concentration-dependent mode of gluconate uptake is indicated also by the induction of enzymes of gluconate catabolism (table 2). Whereas the wild-

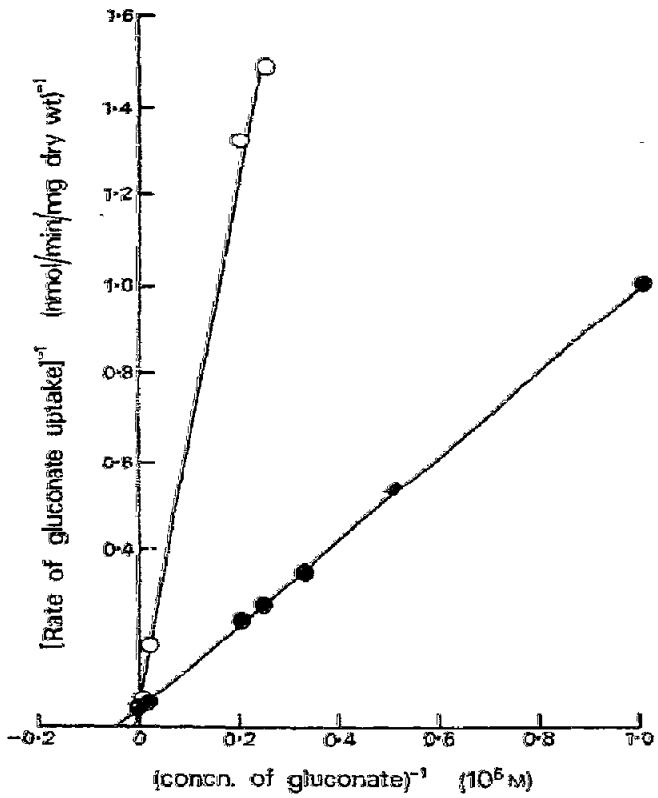

Fig. 2. Effect of gluconate concentration on the rates of gluconate uptake by washed suspensions of gluconate-grown *E. coli* strains P4X (•) and PF1 (○). The rates of uptake were calculated from the amounts of ^{14}C incorporated by washed suspensions of cells (0.34 mg dry wt) in the first 30 sec of incubation with the isotopic material; the reciprocals of these rates are plotted against the reciprocals of the gluconate concentration at those points.

type strain P4X, which forms little gluconate kinase and 6-phosphogluconate dehydratase in the absence of gluconate, inducibly synthesizes both enzymes during growth on glycerol in the presence of gluconate, or on gluconate alone, the mutant PF1 forms only the dehydratase in normal amounts under these conditions: although gluconate kinase activity is induced to high levels, its specific activity does not exceed half that observed with wild-type cells, even after prolonged growth on gluconate. On the other hand, the variations with growth substrate in the levels of other enzymes of gluconate catabolism are not grossly dissimilar in mutant and wild-type cells. These observations support the view [2,4] that *E. coli* contains two alleles that specify transport systems, and two

Table 2
Effect of growth substrates on intracellular levels of enzymes of gluconate catabolism.

Carbon source for growth	Organism	Specific activity* of: Gluconate kinase	6-Phospho-gluconate dehydratase**	Phospho 2-keto 3-deoxygluconate aldolase	6-Phospho-gluconate dehydrogenase
20 mM Glycerol	P4X	< 1	20	250	78
	PF1	< 1	27	290	90
20 mM Glycerol plus 5 mM gluconate	P4X	110	150	780	95
	PF1	50	110	500	86
20 mM Gluconate	P4X	130	300	1100	120
	PF1	70	330	680	70

The methods used for assay of these enzymes are as described by Pouysségur and Stoeber [21].

* nmoles·min^{-1}·mg^{-1} of protein
** Calculated from dry wt. of cells used.

that specify kinase activity; it would appear that mutant PF1 is impaired in a region of the genome that governs the formation both of one (high-affinity, low K_m) uptake system and one form of gluconate kinase. This pleiotropic lesion might be associated with a polar mutation or a small deletion: the extraordinarily low reversion rate of mutant PF1 favours the latter.

3.3. *Location of the* usgA*-marker on the E coli genome*

The Hfr-strain PF1 was crossed with a recipient strain A1201, recombinants were selected for the utilization of appropriate sugars or for the loss of auxotrophic markers, and these were tested for gluconate uptake by the film technique. It was found that, of 50 recombinants able to grow on xylose, 18 had acquired the UsgA$^-$-phenotype, whereas 58 out of 76 recombinants selected for growth on maltose had done so. Analysis of one *usgA*-recombinant still unable to utilize maltose or xylose supported the location of the *usgA*-marker between those specifying response to streptomycin (64 min on the linkage map [18]) and the ability to grow on xylose (70 min), and close to the *malA*-marker (66 min). This location was confirmed by transduction in which phage P1, grown on a recombinant carrying the *usgA* and *xyl* markers, infected a recipient carrying *malA* and a deletion known to be 30% co-transducible with *xyl* [19] of the *dct*-allele [20]. Of the transductants phenotypically Maltose$^+$, about a quarter were impaired in gluconate uptake; in contrast, none of the 210 transductants selected for growth on C_4-acids (and hence *dct*$^+$) were affected in gluconate uptake. It is thus probable that the high-affinity uptake system for gluconate specified by the *usgA*-allele is located at about 66 min on the *E coli* linkage map, close to the *malA* marker. It is also evident that the properties of mutant PF1, its ability apparently inducibly to form a second (low-affinity) uptake system for gluconate, and the genetic location of the *usgA*-marker, are so closely similar to those briefly reported by de Zwaig et al. [2] as to make it likely that they are mutants of the same system.

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