Glucose and Gluconate Metabolism in a Mutant of Escherichia coli Lacking Gluconate-6-phosphate Dehydrase

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A mutant lacking gluconate-6-phosphate dehydrase (the first enzyme of the Entner-Doudoroff pathway) was isolated after ethyl methane sulfonate mutagenesis of Escherichia coli. Other enzymes of gluconate metabolism (gluco- kinase, gluconate-6-phosphate dehydrogenase, and 2-keto-3-deoxygluconate-6-phosphate aldolase) were present in the mutant. When the mutant was grown on gluconate-$\text{H}_2\text{C}_6\text{O}_7$-1$\text{4}$C, alanine isolated from protein was unlabeled, showing that the dehydrase was absent in vivo and that the sole pathway of gluconate metabolism in the mutant was the hexose monophosphate shunt. The mutant grew on gluconate with a doubling time of 155 min, compared with the parent strain's 56 min. On glucose and fructose it grew with normal doubling times. Thus, in E. coli, the Entner-Doudoroff pathway is used for gluconate metabolism but not for glucose metabolism.

Pathways of gluconate metabolism in Enterobacteriaceae are shown in Fig. 1. Glucuronate is phosphorylated by an inducible kinase (1). The product, gluconate-6-phosphate, is partly used by the pentose phosphate pathway, which might both supply pentose for nucleic acid and, acting as the hexose monophosphate shunt, form fructose-6-phosphate. Glucuronate-6-phosphate is principally used, however, by the Entner-Doudoroff pathway (3), which involves an inducible glucuronate-6-phosphate dehydrase, forming 2-keto-3-deoxygluconate-6-phosphate (KDG), which is cleaved by KDG aldolase, giving pyruvate and glyceraldehyde-3-phosphate. This scheme is based on data gathered from experiments on growth rates, enzyme content, and labeling patterns in phosphoglucone isomerase-negative mutants and their parent strains, both in Salmonella typhimurium (5) and in Escherichia coli (6). To date, however, there have been no reports of mutants blocked in gluconate metabolism. In this paper, we describe the isolation and properties of one such mutant of E. coli, whose properties provide independent verification of these pathways.

Materials and Methods

Most methods and assays used in this work are described in the accompanying papers (4, 6). Glucuronate-6-phosphate dehydrase was always assayed in extracts prepared from logarithmically growing cells; extracts from cultures harvested in stationary phase often have appreciably lower activities of this enzyme. Since it is an inducible enzyme and growth of some mutants may be slow in gluconate minimal medium, it is convenient when assaying strains for this enzyme to harvest cultures from logarithmic growth in a gluconate-containing broth, which is minimal medium 63 supplemented with 1% tryptone (Difco) and 0.4% sodium gluconate. Gluconate-tetrazolium plates were like lactose-tetrazolium plates (8) but contained 1% sodium gluconate instead of 1% lactose.

Results

Selection of mutant. Strain K-10, 2.5 $\times$ 10$^8$ viable cells per ml, in medium 63 was treated with 0.035 volumes of ethyl methane sulfonate (7) for 20 min at 37 C, and dilutions were plated on gluconate-tetrazolium plates. (On such plates, wild-type \textquotedblleft fermentionings\textquotedblright give pale colonies, whereas mutants are varying shades of red.) The mutagenic treatment reduced the viable count to $9 \times 10^7$. Of 1,081 colonies arising from a nonmutagenized culture, only 1 was red, whereas 10 of 731 colonies after mutagenesis were red. These were purified and tested on minimal media. None of them was an absolute gluconate-negative strain. Six formed colonies of almost normal size on gluconate, three formed tiny colonies on several carbon sources, and one strain, RZ10 (which arose from the mutagenized culture), formed normal-sized colonies on glucose (ca. 2 mm in diameter at 48 hr of incubation) and small colonies (ca. 0.4 mm) on gluconate.

Enzyme activities. The activity of some enzymes
of gluconate metabolism in K-10 and RZ10 is given in Table 1. When grown in gluconate-containing broth, RZ10 contained gluconokinase, gluconate - 6 - phosphate dehydrogenase, and KDGP aldolase, but there was no detectable gluconate-6-phosphate dehydrase. Table 1 also shows the activities of these enzymes in RZ10 after growth in glucose and in gluconate minimal medium. Again, RZ10 lacked dehydrase activity, and, as in the parent strain (6), gluconokinase was inducible and gluconate-6-phosphate dehydrogenase and KDGP aldolase were relatively unaffected by the presence of gluconate in the growth medium.

**Growth rates.** The growth rates of RZ10 and K-10 in several media are given in Table 2. The mutant grew at normal rates on glucose and on fructose, whereas on gluconate it grew about one-third as fast as the parent strain.

**Isotopic experiment.** According to the scheme of Fig. 1, a mutant lacking gluconate-6-phosphate dehydrase would be expected to use gluconate exclusively via gluconate-6-phosphate dehydrogenase and the pentose phosphate pathway. The utilization of gluconate-1-14C, then, would involve the loss of the labeled carbon atom in a decarboxylation, and a metabolite such as pyruvate would be unlabeled. We have indirectly assayed the specific activity of pyruvate during growth of RZ10 on gluconate-1-14C by isolating and degrading alanine from protein after growth in this medium. [The details of these experiments are described in the accompanying paper (6)]. When the gluconate-1-14C had a specific activity of 4.27 × 104 dpm per µmole, the isolated alanine had an activity of 0.01 × 104 dpm per µmole—essentially unlabeled. Hence, the gluconate-6-phosphate dehydrase is absent in vivo as well as in extracts.

**Mapping.** The genetic locus for gluconate-6-

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**Table 1. Enzymes of gluconate metabolism**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Parent (K-10), gluconate broth</th>
<th>Mutant (RZ10), gluconate broth</th>
<th>Mutant (RZ10), Glucose minimal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gluconokinase...</td>
<td>189+</td>
<td>72</td>
<td>51</td>
</tr>
<tr>
<td>Gluconate-6-phosphate dehydrogenase...</td>
<td>180</td>
<td>137</td>
<td>132</td>
</tr>
<tr>
<td>Gluconate 6-phosphate dehydrase...</td>
<td>180</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>KDGP aldolase...</td>
<td>338</td>
<td>342</td>
<td>203</td>
</tr>
</tbody>
</table>

* Enzyme activity expressed as millimicromoles per minute per milligram of protein.

**Table 2. Growth rates in minimal medium**

<table>
<thead>
<tr>
<th>Medium</th>
<th>Parent (K-10)</th>
<th>Mutant (RZ10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>60</td>
<td>61</td>
</tr>
<tr>
<td>Fructose</td>
<td>79</td>
<td>75</td>
</tr>
<tr>
<td>Gluconate</td>
<td>56</td>
<td>155</td>
</tr>
</tbody>
</table>

phosphate dehydrase will be assigned the provisional name edd (Entner-Doudoroff dehydrase) and the mutation in RZ10 is edd-1 [as suggested by Demerec et al. (2)]. In genetic crosses, edd-1 segregates as a single marker. RZ10, being derived from K-10, is a prototrophic, streptomycin-sensitive Hfr strain injecting in the order origin leu metB str argG his. It was mated with JC411 (leu-, metB-, str-, argG+, his-), and amino acid-positive recombinants were selected on streptomycin-containing plates. These recombinants were tested for edd by spotting onto gluconate-tetrazolium plates. There were no edd- out of 60 leu- recombinants, 6 edd- per 100 met-, 9 edd- per 100 arg-, and 67 edd- per 100 his-.

This and similar crosses with other F- strains place the edd locus in the vicinity of his; more accurate mapping experiments are in progress.

**DISCUSSION**

RZ10 lacks both in vitro and in vivo activity of gluconate-6-phosphate dehydrase, as shown by enzyme assays and the labeling experiment. As is shown in Fig. 2, RZ10 is analogous with the phosphoglucose isomerase-negative mutant L40 (6); each has lost a major pathway for a carbon source (glucose for L40, gluconate for RZ10), and the metabolism of that carbon source seems...
GLUCONATE-6-PHOSPHATE DEHYDRASE MUTANT

FIG. 2 Comparison of two mutants. Outline of glucose and gluconate pathways showing the blocks in L40 and RZ10.

restricted to the hexose monophosphate shunt. Indeed, the growth rates are similar: L40 on glucose, 168 min (6); RZ10 on gluconate, 155 min. As was discussed in connection with L40 (6), it is not known whether growth of RZ10 on gluconate is slow solely because some enzyme in the shunt is rate-limiting.

Since RZ10 has both phosphoglucose isomerase and a hexose monophosphate shunt, one would expect its glucose metabolism to be normal. Indeed, it does grow on glucose at the same rate as the parent strain K-10. Thus, these experiments with RZ10 confirm other work (7; Eisenberg and Dobrogosz, Bacteriol. Proc., 1966, p. 77): the Entner-Doudoroff pathway has a major role in gluconate metabolism, but little, if any, role in normal glucose metabolism in E. coli.

ACKNOWLEDGMENTS

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LITERATURE CITED