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The Entner-Doudoroff Pathway in *Escherichia coli* Is Induced for Oxidative Glucose Metabolism via Pyrroloquinoline Quinone-Dependent Glucose Dehydrogenase

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The Entner-Doudoroff pathway was shown to be induced for oxidative glucose metabolism when *Escherichia coli* was provided with the periplasmic glucose dehydrogenase cofactor pyrroloquinoline quinone (PQQ). Induction of the Entner-Doudoroff pathway by glucose plus PQQ was established both genetically and biochemically and was shown to occur in glucose transport mutants, as well as in wild-type *E. coli*. These data complete the body of evidence that proves the existence of a pathway for oxidative glucose metabolism in *E. coli*. PQQ-dependent oxidative glucose metabolism provides a metabolic branch point in the periplasm; the choices are either oxidation to gluconate followed by induction of the Entner-Doudoroff pathway or phosphotransferase-mediated transport. The oxidative glucose pathway might be important for survival of enteric bacteria in aerobic, low-phosphate, aquatic environments.

MATERIALS AND METHODS

**Bacterial strains, plasmids, and growth conditions.** The glucose transport mutant used in this study was *E. coli* ZSC113 (*ptsM12 ptsG22 glk*) (9). The wild-type strain used in this study was *E. coli* W3110 (2). These strains were routinely grown in complex medium (Luria broth [18]) with or without added carbohydrate (0.5%) at 37°C. Minimal MOPS (morpholinepropanesulfonic acid) medium contained 50 mM MOPS (pH 6.8), 15 mM (NH$_4$)$_2$SO$_4$, 5 mM KH$_2$PO$_4$, 1.8 μM FeSO$_4$, 1 mM MgSO$_4$, and 0.05 mg of thiamine per ml. All cultures used for experiments, particularly those containing PQQ (1 mg/liter), were pregrown in the same medium prior to inoculation and were harvested in mid-logarithmic phase. Carbohydrates were filter sterilized and added to the basal medium at final concentration of 0.4%.

**Enzyme assays.** *E. coli* cells were prepared for enzyme assays as described previously (3). 6-Phosphogluconate dehydratase was assayed by previously described methods (10, 14). 2-Keto-3-deoxy-6-phosphogluconate (KDPG) aldolase was assayed as described previously (7).

**RNA isolation and analysis.** RNA isolations were conducted as described previously (15). A Northern (RNA) blot analysis was carried out as described previously (8) by using DNA hybridization probes that were labeled with a random primed labeling kit. An *edd*-specific DNA hybridization probe was prepared as a 1.14-kb *BstEII* restriction fragment from pTC180. An *eda*-specific probe was prepared from pTC196 as a 0.28-kb *HincII*-to-*BamHI* restriction fragment. Determinations of RNA sizes were based on the migration distances of known RNA standards, as described previously (15).

**Enzymes and chemicals.** Restriction enzymes and DNA-modifying enzymes were obtained from Bethesda Research Laboratories, Inc., Gaithersburg, Md. The random primed DNA labeling kit was ordered from U.S. Biochemical Corp., Cleveland, Ohio. Radioactive compounds were purchased from New England Nuclear Corp., Boston, Mass. Biochemicals were obtained from Sigma Chemical Co., St. Louis, Mo.
TABLE 1. 6-Phosphogluconate dehydratase and KDPG aldolase activities in E. coli ZSC113

<table>
<thead>
<tr>
<th>Medium</th>
<th>Carbon source(s)</th>
<th>Mean enzyme activities (SD)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>6-Phosphogluconate dehydratase</td>
</tr>
<tr>
<td>Minimal</td>
<td>Glucose + PQQ</td>
<td>14.4 (1.5)</td>
</tr>
<tr>
<td>Minimal</td>
<td>Glucuronate</td>
<td>40.6 (2.0)</td>
</tr>
<tr>
<td>Luria broth</td>
<td>None</td>
<td>1.6 (0.2)</td>
</tr>
</tbody>
</table>

* Enzyme activities are expressed as nanomoles per minute per milligram of total cell protein.

RESULTS

PQQ stimulates oxidative glucose metabolism in a glucose transport mutant. Several recent reports have shown that E. coli is capable of oxidative glucose metabolism via periplasmic glucose dehydrogenase if cofactor PQQ is available (1, 4, 5). It was hypothesized that glucose formed in the periplasm by oxidation of glucose leads to induction of the Entner-Doudoroff pathway. E. coli ZSC113 is a phosphotransferase transport-defective mutant (9) that is not able to grow on glucose except when PQQ is added (1). Table 1 shows that the enzymes of the Entner-Doudoroff pathway were induced under these conditions. Growth of E. coli ZSC113 on minimal containing glucose plus PQQ resulted in a ninefold induction of 6-phosphogluconate dehydratase compared with the same strain grown on Luria broth without added carbohydrates. This value compares with a 25-fold induction of 6-phosphogluconate dehydratase for E. coli ZSC113 grown on glucose. Thus, the level of 6-phosphogluconate dehydratase induced by growth on medium containing glucose plus PQQ was only one-third of the fully induced level in cells grown on glucose.

PQQ stimulates oxidative glucose metabolism in wild-type E. coli. Since induction of the Entner-Doudoroff pathway is evidently not subject to catabolite repression by glucose (Table 1), it seemed reasonable to predict that wild-type E. coli would be capable of utilizing the Entner-Doudoroff pathway for oxidative glucose metabolism in the presence of PQQ. Table 2 shows that this is clearly the case. Growth of E. coli W3110 on minimal containing glucose plus PQQ caused induction of 6-phosphogluconate dehydratase activity equivalent to induction of E. coli ZSC113. The level of 6-phosphogluconate dehydratase induced by glucose plus PQQ was one-third the level induced by glucose.

We have recently shown that induction of the Entner-Doudoroff pathway occurs at the transcriptional level; growth of E. coli DH5α on gluanonate resulted in formation of a 2.6-kb transcript that encoded the edd and eda genes (10). Transcriptional induction of the Entner-Doudoroff pathway in E. coli W3110 was confirmed by a Northern blot analysis. Transcription of a 2.6-kb message that could be detected with either the edd- or eda-specific hybridization probes was induced by growth on medium containing glucose plus PQQ, glucuronate, or glucose plus glucuronate, but not by growth on medium containing glucose or glucuronate alone (Fig. 1). Induction of the edd-eda polycistrionic mRNA was paralleled by corresponding increases in 6-phosphogluconate dehydratase activity. The 1.0- and 0.75-kb, eda-specific transcripts were present under all five growth conditions (Fig. 1). The ratio of the 0.75-kb transcript to the 1.0-kb transcript increased when E. coli W3110 was grown on medium containing glucose plus PQQ, glucuronate, or glucose plus glucuronate, but not when it was grown on medium containing only glucose or glucuronate. Thus, the 3.5-fold induction of KDPG aldolase caused by growth on glucuronate (Tables 1 and 2) appears to be the result of increased transcription of the 0.75-kb mRNA. There appeared to be a modest increase in the relative abundance of the 1.0-kb transcript when the organism was grown on glucuronate, but not when it was grown on the other carbon sources (Fig. 1). These data confirm the twofold induction of KDPG aldolase activity for E. coli W3110 grown on glucuronate (Table 2) and the previously observed threefold induction of KDPG aldolase activity for growth of another E. coli K-12 strain on glucuronate (22). Furthermore, the data suggest that P₂ (eda

TABLE 2. 6-Phosphogluconate dehydratase and KDPG aldolase activities in E. coli W3110

<table>
<thead>
<tr>
<th>Medium</th>
<th>Carbon source(s)</th>
<th>Mean enzyme activities (SD)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>6-Phosphogluconate dehydratase</td>
</tr>
<tr>
<td>Minimal</td>
<td>Glucose</td>
<td>4.3 (1.0)</td>
</tr>
<tr>
<td>Minimal</td>
<td>Glucuronate</td>
<td>43.8 (5.7)</td>
</tr>
<tr>
<td>Minimal</td>
<td>Glucuronate</td>
<td>2.8 (1.4)</td>
</tr>
<tr>
<td>Minimal</td>
<td>Glucose + glucuronate</td>
<td>30.7 (3.7)</td>
</tr>
<tr>
<td>Minimal</td>
<td>Glucose + PQQ</td>
<td>14.8 (0.3)</td>
</tr>
<tr>
<td>Low PO₄*</td>
<td>Glucose</td>
<td>5.5 (0.4)</td>
</tr>
<tr>
<td>Low PO₄</td>
<td>Glucuronate</td>
<td>31.4 (0.9)</td>
</tr>
<tr>
<td>Low PO₄</td>
<td>Glucuronate</td>
<td>3.8 (0.3)</td>
</tr>
<tr>
<td>Low PO₄</td>
<td>Glucose + glucuronate</td>
<td>24.0 (1.3)</td>
</tr>
<tr>
<td>Low PO₄</td>
<td>Glucose + PQQ</td>
<td>12.9 (0.5)</td>
</tr>
</tbody>
</table>

* Enzyme activities are expressed as nanomoles per minute per milligram of total cell protein.
* Minimal MOPS medium containing 10 μM potassium phosphate.
way. The catabolism would be catabolite in the has indicated and gluconate via the transport catabolism. This of metabolism.

Entner-Doudoroff pathway is not induced by phosphate limitation. The results of previous work in our laboratories suggested that uptake of PQO into the periplasm is stimulated by induction of the PhoE porin under phosphate-limiting conditions (1). A recent update of the E. coli gene-protein data base indicated that the spot on two-dimensional gels corresponding to KDPG aldolase was among the 82 phosphate starvation-inducible proteins (24). In order to determine whether phosphate limitation could lead to induction of the Entner-Doudoroff pathway, the enzymes of the pathway were assayed under phosphate-limiting conditions. E. coli W3110 was grown in minimal medium containing 10 μM phosphate; conditions that are known to induce a large number of phosphate starvation proteins (24). However, growth on phosphate-limited glucose-containing medium did not lead to induction of 6-phosphogluconate dehydratase; the extents of dehydratase induction were similar in the presence of high and low concentrations of phosphate with all of the carbon sources tested (Table 2). The KDPG aldolase activities were also similar in the presence of high and low concentrations of phosphate with all of the carbon sources tested (Table 2). It should be noted that the phosphate-inducible starvation spot designated KDPG aldolase in a previous two-dimensional gel analysis (24) was actually composed of two proteins; presumably, it is the other protein that is actually phosphate starvation inducible, although it is possible that KDPG aldolase is induced by phosphate starvation but not by phosphate limitation.

**DISCUSSION**

Although it has been a matter of speculation for some time (1, 13, 16), on the basis of the evidence outlined above it is now clear that E. coli possesses a pathway for oxidative glucose metabolism. In the presence of PQO, the glucose dehydrogenase apoenzyme becomes functional, resulting in formation of gluconate from glucose in the periplasm (5, 16). This represents a metabolic branch point in the periplasm for glucose catabolism. The alternative branches consist of (i) phosphotransferase-mediated transport or (ii) oxidation to gluconate and subsequent transport, phosphorylation, and catabolism via the gluconate-inducible Entner-Doudoroff pathway. Simultaneous use of both of these pathways for glucose catabolism would be possible only in the absence of glucose catabolite repression of the Entner-Doudoroff pathway. The absence of such catabolite repression was clearly indicated by the results of this study.

Growth of E. coli on medium containing glucose plus PQO has been shown to result in extracellular gluconate concentrations in the micromolar range (4). Whether gluconate itself or a subsequent metabolic intermediate is the signal for induction is not known for certain. One possible candidate for an inducer molecule is 6-phosphogluconate. However, increased intracellular concentrations of 6-phosphogluconate, which would be expected to build up in E. coli phosphoglucone isomerase mutants when they are growing on glucose, did not cause induction of the Entner-Doudoroff pathway (14). Likewise, E. coli strains that lack phosphoglucone isomerase and 6-phosphogluconate dehydrogenase did not grow on glucose (17). Interestingly, E. coli 6-phosphogluconolactonase mutants (17), as well as phosphoglucone isomerase–6-phosphogluconate dehydrogenase–fructose diphosphate aldolase triple mutants (23), were able to grow on glucose via induction of the Entner-Doudoroff pathway. In these cases it appears that the metabolic lesions led to leakage of intracellular 6-phosphogluconolactone, which was dephosphorylated and hydrolyzed to cause accumulation of gluconate in the periplasm, thus leading to induction of the Entner-Doudoroff pathway (17, 23). Our results are consistent with the notion that gluconate itself is the inducer of the Entner-Doudoroff pathway.

With the realization that there is a PQO-dependent glucose branch point in the periplasm come questions regarding the role of oxidative glucose metabolism in nature. In a previous study, we showed that the oxidative glucose pathway is utilized only under aerobic conditions (1). Furthermore, low phosphate concentrations in the growth medium greatly reduced the lag phase for growth on medium containing glucose plus PQO, suggesting that induction of the PhoE porin facilitated PQO uptake into the periplasm (1). The nature of induction of this pathway in enteric bacteria suggests an important role in aerobic aquatic habitats which contain free PQO and low carbon and phosphate concentrations (19). Perhaps this pathway allows survival during the extraintestinal, aquatic phases of E. coli's existence. Limiting phosphate concentrations in aquatic environments would result in uptake of PQO, turning on glucose dehydrogenase and glucose oxidation to gluconate. Thus, phosphate limitation would lead indirectly to induction of the Entner-Doudoroff pathway by the gluconate derived from glucose. Oxidative glucose metabolism, as opposed to phosphotransferase transport and glycolysis, may indeed provide a bioenergetic advantage in the environment, since it is found in a large number of enteric and free-living, aquatic microorganisms (25). It seems reasonable that E. coli should be able to harness the energy of electron transfer via PQO-dependent glucose dehydrogenase (25). In this regard, it should be noted that growth on medium containing glucose plus PQO resulted in higher cell yields than growth on medium containing glucose alone (1).

**ACKNOWLEDGMENTS**

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