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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.

The Entner-Doudoroff pathway (6, 12) forms the core of central metabolism in many bacteria, including *Zymomonas mobilis* (26) and *Pseudomonas aeruginosa* (21). However, the physiological role of the Entner-Doudoroff pathway in *Escherichia coli* is still unclear (13). The discovery of a pyrroloquinoline quinone (PQQ)-dependent glucose dehydrogenase, which catalyzes the oxidation of glucose to gluconic acid in the periplasm, suggested an alternate route for glucose catabolism in *E. coli* (16). Mutants defective in enzyme I of the phosphotransferase system were able to grow on glucose in the presence of exogenous PQQ, the cofactor for the glucose dehydrogenase apoenzyme (16). Paradoxically, wild-type *E. coli* does not synthesize PQQ (16). The *E. coli* glucose dehydrogenase was subsequently cloned, and the gene was sequenced (5). Data from our laboratories suggested that in the presence of functional glucose dehydrogenase, glucose could be converted to gluconate in the periplasm, leading to induction of the Entner-Doudoroff pathway for catabolism of the gluconate (1). More recently, *E. coli* mutants capable of producing PQQ were isolated, and the hypothesis that a pathway for oxidative glucose metabolism exists was supported (4). However, formal proof that oxidative glucose metabolism occurs via the Entner-Doudoroff pathway has not been reported. That is, can the Entner-Doudoroff enzymes be turned on by the simultaneous presence of glucose and PQQ? In this paper we provide direct evidence that the Entner-Doudoroff pathway is turned on by oxidation of glucose to gluconate in the periplasm. In addition, the role of limiting phosphate in regulating the Entner-Doudoroff pathway was examined in this study. It is concluded that for *E. coli*, a low phosphate concentration promotes use of the Entner-Doudoroff pathway indirectly by providing access of PQQ into the periplasm rather than directly by derepressing *edd* and *eda*.

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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 $(NH_4)_2SO_4$, 5 mM KH_2PO_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 *Bst*EII restriction fragment from pTC180. An *eda*-specific probe was prepared from pTC196 as a 0.28-kb *Hin*cII-to-*Bam*HI 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

Medium	Carbon source(s)	Mean enzyme activities (SD)[a]	
		6-Phosphogluconate dehydratase	KDPG aldolase
Minimal	Glucose + PQQ	14.4 (1.5)	296 (36)
Minimal	Gluconate	40.6 (2.0)	1,166 (92)
Luria broth	None	1.6 (0.2)	156 (16)

[a] 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 gluconate 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 medium 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 gluconate. 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 gluconate.

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 medium containing glucose plus PQQ caused induction of 6-phosphogluconate dehydratase activity equivalent to induction of *E. coli* ZSC113. The level of

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

Medium	Carbon source(s)	Mean enzyme activities (SD)[a]	
		6-Phosphogluconate dehydratase	KDPG aldolase
Minimal	Glucose	4.3 (1.0)	163 (14)
Minimal	Gluconate	43.8 (5.7)	587 (23)
Minimal	Glucuronate	2.8 (1.4)	302 (42)
Minimal	Glucose + gluconate	30.7 (3.7)	519 (28)
Minimal	Glucose + PQQ	14.8 (0.3)	319 (24)
Low PO_4[b]	Glucose	5.5 (0.4)	103 (12)
Low PO_4	Gluconate	31.4 (0.9)	448 (32)
Low PO_4	Glucuronate	3.8 (0.3)	306 (23)
Low PO_4	Glucose + gluconate	24.0 (1.3)	401 (34)
Low PO_4	Glucose + PQQ	12.9 (0.5)	219 (26)

[a] Enzyme activities are expressed as nanomoles per minute per milligram of total cell protein.
[b] Minimal MOPS medium containing 10 μM potassium phosphate.

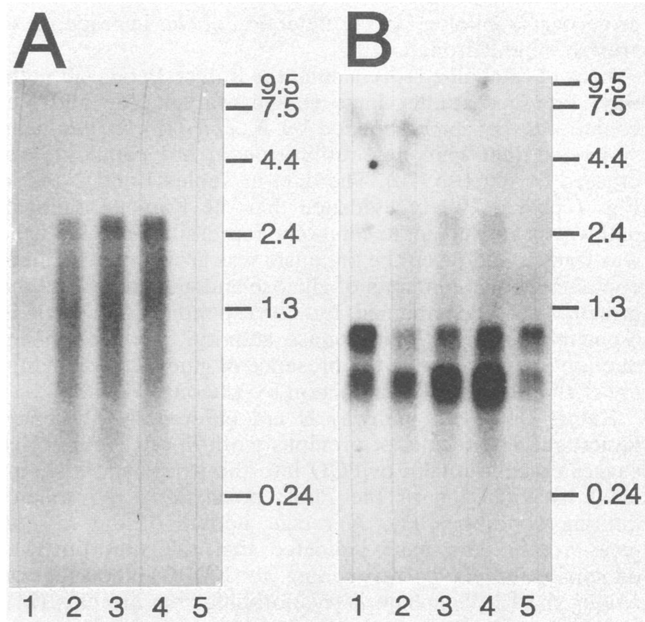

FIG. 1. Northern blot analysis of *edd-eda* expression in *E. coli* W3110 grown in minimal medium. Total RNA was harvested from log-phase cultures grown in minimal medium containing glucuronate (lanes 1), glucose plus PQQ (lanes 2), glucose plus gluconate (lanes 3), gluconate (lanes 4), or glucose (lanes 5). The gels were loaded with 2.5 μg of RNA per lane. The positions of RNA size standards are shown on the right of both panels. (A) RNA hybridized with an *edd*-specific probe. (B) RNA hybridized with an *eda*-specific probe.

6-phosphogluconate dehydratase induced by glucose plus PQQ was one-third the level induced by gluconate.

We have recently shown that induction of the Entner-Doudoroff pathway occurs at the transcriptional level; growth of *E. coli* DH5α on gluconate 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, gluconate, or glucose plus gluconate, but not by growth on medium containing glucose or glucuronate alone (Fig. 1). Induction of the *edd-eda* polycistronic 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, gluconate, or glucose plus gluconate, 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 gluconate (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 P_2 (*eda* promoter) is involved in the induction of *eda* in response to growth on glucuronate (10).

Lack of catabolite repression of the Entner-Doudoroff pathway. Previous studies have shown that glucose and gluconate can be cometabolized by *E. coli* (11). It has been suggested that gluconate utilization is not catabolite repressed by glucose (20). The data in Tables 1 and 2 and in Fig. 1 provide direct evidence that the Entner-Doudoroff pathway is not subject to catabolite repression. The *edd* gene was transcribed when the organism was grown on a medium containing equal amounts of glucose and gluconate, and the growth was accompanied by corresponding increases in 6-phosphogluconate dehydratase activity. The level of induction by gluconate in the presence of glucose was slightly lower than the level of induction by gluconate alone.

Entner-Doudoroff pathway is not induced by phosphate limitation. The results of previous work in our laboratories suggested that uptake of PQQ 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 PQQ, 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 PQQ 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* phosphoglucose isomerase mutants when they are growing on glucose, did not cause induction of the Entner-Doudoroff pathway (14). Likewise, *E. coli* strains that lack phosphoglucose isomerase and 6-phosphogluconate dehydrogenase did not grow on glucose (17). Interestingly, *E. coli* 6-phosphogluconolactonase mutants (17), as well as phosphoglucose 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 PQQ-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 PQQ, suggesting that induction of the PhoE porin facilitated PQQ 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 PQQ 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 PQQ, 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 PQQ-dependent glucose dehydrogenase (25). In this regard, it should be noted that growth on medium containing glucose plus PQQ resulted in higher cell yields than growth on medium containing glucose alone (1).

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