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Nutritional Complementation of Oxidative Glucose Metabolism in *Escherichia coli* via Pyrroloquinoline Quinone-Dependent Glucose Dehydrogenase and the Entner-Doudoroff Pathway

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Two glucose-negative *Escherichia coli* mutants (ZSC113 and DF214) were unable to grow on glucose as the sole carbon source unless supplemented with pyrroloquinoline quinone (PQQ). PQQ is the cofactor for the periplasmic enzyme glucose dehydrogenase, which converts glucose to gluconate. Aerobically, *E. coli* ZSC113 grew on glucose plus PQQ with a generation time of 65 min, a generation time about the same as that for wild-type *E. coli* in a defined glucose-salts medium. Thus, for *E. coli* ZSC113 the Entner-Doudoroff pathway was fully able to replace the Embden-Meyerhof-Parnas pathway. In the presence of 5% sodium dodecyl sulfate, PQQ no longer acted as a growth factor. Sodium dodecyl sulfate inhibited the formation of gluconate from glucose but not gluconate metabolism. Adaptation to PQQ-dependent growth exhibited long lag periods, except under low-phosphate conditions, in which the PhoE porin would be expressed. We suggest that *E. coli* has maintained the apoenzyme for glucose dehydrogenase and the Entner-Doudoroff pathway as adaptations to an aerobic, low-phosphate, and low-detergent aquatic environment.

Although *Escherichia coli* is commonly considered to be an intestinal bacterium, it is also well adapted for aquatic environments. *E. coli* is able to metabolize a wide range of carbohydrate substrates via the Embden-Meyerhof-Parnas and pentose phosphate pathways, and the recent finding of a pyrroloquinoline quinone (PQQ)-dependent glucose dehydrogenase (6) has suggested a role for oxidative glucose metabolism as well. This pathway, as typified by its use in *Pseudomonas aeruginosa*, involves periplasmic oxidation of glucose to gluconate, followed by uptake and metabolism of that gluconate by the Entner-Doudoroff (ED) pathway. In *E. coli*, the ED pathway is inducible only by extracellular gluconate. Thus, in wild-type strains, no other sugars are metabolized via the ED pathway (9, 11, 24). However, some mutants blocked in their normal glucose catabolic pathways can metabolize glucose via the ED pathway by first converting glucose to extracellular gluconate (17, 21).

Bacteria have the ability to grow in many harsh environments. Among the factors in such environments are extremes of temperature, pH, pressure, and salt and detergent concentrations. We have been studying the ability of many enteric bacteria to tolerate high concentrations of sodium dodecyl sulfate (SDS) (14–16). Recently, we used two-dimensional gel electrophoresis to demonstrate the presence of several "detergent shock proteins" (1). The physiological relevance of these detergent (SDS) shock proteins probably derives from the presence of bile salts in animal gastrointestinal tracts. Evidence for the existence of a class of detergent shock proteins inevitably raises questions regarding their function and location. In this light, we have chosen to focus on the cellular adaptations necessary to cope with the possible presence of SDS in the periplasm of gram-negative bacteria. In particular, we were interested in the in vivo SDS sensitivity of PQQ-dependent glucose dehydrogenase (4, 8). This periplasmic enzyme converts glucose to gluconic acid, which is then transported into the cell and further metabolized via the pentose phosphate and ED pathways (Fig. 1). Paradoxically, *E. coli* appears to be able to use PQQ but not to synthesize it (8). The PQQ-dependent glucose dehydrogenase gene from *E. coli* has recently been cloned and sequenced (6).

We studied two glucose-negative *E. coli* mutants; their growth on glucose was totally dependent on exogenous PQQ and the PQQ-dependent glucose dehydrogenase. The present paper demonstrates the SDS sensitivity of periplasmic glucose dehydrogenase. Additionally, our results pertain to the physiological and ecological significance of PQQ and the ED pathway. For *E. coli*, they would appear to be of selective advantage primarily in an aerobic, low-phosphate, and low-detergent aquatic environment.

MATERIALS AND METHODS

Organisms. The glucose-negative mutants were obtained from B. J. Bachmann, *E. coli* Genetic Stock Center, Yale University, New Haven, Conn. *E. coli* ZSC113 (CGSC 5457) was isolated by Curtis and Epstein (7). It is a triple mutant (*ptsM12 ptsG22 glk-7*) with defects in glucokinase and the phosphotransferase systems (PTS) for both glucose and mannose. It is unable to metabolize glucose by any pathway requiring direct phosphorylation of glucose. We confirmed the expected mutational defects in strain ZSC113 by enzyme assay for glucokinase and by the appearance of white colonies on glucose-MacConkey agar and mannose-MacConkey agar. *E. coli* DF214 (CGSC 5413) was isolated by Vinopal et al. (23). Strain DF214 [Δ(*eda-zwf*)*15 hisG1 pgi-7*::Mu-1], because of blocks in *pgi* and *zwf*, cannot metabolize glucose via the Embden-Meyerhof-Parnas, pentose phosphate, or ED pathways or convert it to 6-phosphogluconate. *E. coli* W3110 was obtained from F. C. Neidhardt.

Medium and aerobic growth conditions. Experiments on the role of PQQ were conducted in minimal medium containing (per liter) 5 g of NaH_2PO_4, 5 g of KH_2PO_4, 2 g of $(NH_4)_2SO_4$, 0.493 g of $MgSO_4 \cdot 7H_2O$, 0.1 g of thiamine, 0.5

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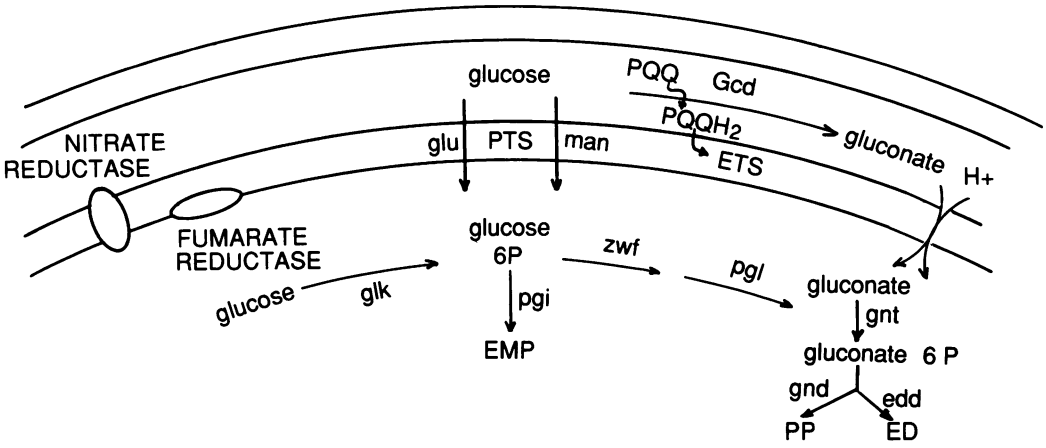

FIG. 1. Metabolic and structural features relevant to SDS resistance in enteric bacteria. Genes: *edd*, 6-phosphogluconate dehydratase (EC 4.2.1.12); *glk*, glucokinase (EC 2.7.1.2); *gnd*, 6-phosphogluconate dehydrogenase (EC 1.1.1.44); *gnt*, gluconokinase (EC 2.7.1.12); *pgi*, phosphoglucoisomerase (EC 5.3.1.9); *pgl*, 6-phosphogluconolactonase (EC 3.1.1.31); *zwf*, glucose 6-phosphate dehydrogenase (EC 1.1.1.49). Other abbreviations: EMP, Embden-Meyerhof-Parnas pathway; ETS, electron transport system; Gcd, PQQ-dependent glucose dehydrogenase; PP, pentose phosphate pathway; glu and man, glucose and mannose (the preferred substrates in the PTS).

mg of $FeSO_4$, and 2 g of the carbon source, adjusted to pH 6.8. Where indicated, SDS was present at 5% (wt/vol) and PQQ was present at 1 μg/ml (3 μM). PQQ, SDS, $MgSO_4 \cdot 7H_2O$, and the carbon source were sterilized separately and added after autoclaving. All cell yields reported are the averages of at least three experiments.

Medium and anaerobic growth conditions. The vitamin-salts medium contained the following per liter of distilled water (pH 6.9): 4.0 g of Na_2HPO_4, 1.0 g of KH_2PO_4, 0.41 g of $MgCl_2 \cdot 6H_2O$, 2.0 g of $(NH_4)_2SO_4$, 0.03 g of $Na_2S_2O_3$, 0.003 g of $FeSO_4$, 0.01 g of Na_2MoO_4, 0.0001 g of riboflavin, and 0.1 g of thiamine. Where indicated, KNO_3 was present at 1%, sodium fumarate was present at 1%, glucose or gluconate was present at 1%, and PQQ was present at 3 μM. Screw-cap culture tubes of ca. 8-ml capacity were overfilled and tightened so that no air bubbles were present. Tubes were incubated at 37°C, and their turbidity was measured directly in a Klett colorimeter with the no. 66 filter. All experiments were repeated with both gluconate-grown and glucose-plus-PQQ-grown *E. coli* ZSC113 inocula.

RESULTS

Aerobic growth of *E. coli* ZSC113. As would be expected for a mutant that is totally deficient for glucose uptake, *E. coli* ZSC113 was unable to grow on a defined liquid medium with glucose as the sole source of carbon and energy (Table 1). However, it did grow well with glucose plus PQQ, gluconate, or a glucose-gluconate mixture (Table 1). The glucose-gluconate mixture was used to rule out the accumulation of toxic metabolites in the mutant. With glucose plus PQQ, growth levels of ca. 200 Klett units were achieved with all PQQ concentrations ≥3 μM. No growth occurred within 48 h with PQQ concentrations ≤1 μM. The growth levels achieved on glucose plus ≥3 μM PQQ were one-third higher than those achieved on gluconate alone or on glucose-gluconate. Presumably (8), this difference reflects the cells ability to use the $PQQH_2$ (reduced PQQ) produced during the glucose-to-gluconate conversion for additional energy production. The higher Klett units for growth in glucose plus PQQ were accompanied by a ca. 40% increase in cell number. Furthermore, the cells which grew in glucose plus PQQ were not glucose-positive revertants; less than one cell in 10^7 formed colonies on agar plates with glucose as the sole carbon source.

SDS sensitivity. PQQ-dependent glucose dehydrogenase functions in the periplasm (8) and should be sensitive to noxious agents that penetrate to this region. In the presence of 5% SDS, PQQ no longer acted as a growth factor (Table 1) but growth on gluconate and on the glucose-gluconate mixture still occurred. Thus, SDS inhibited the formation of gluconate from glucose but not gluconate uptake and metabolism. The decreased cell yields observed for growth in the presence of 5% SDS probably reflect the energy burden observed previously with the growth of *Enterobacter cloacae* in 10% SDS (15).

PQQ-dependent lag periods. *E. coli* ZSC113 inoculated into glucose plus PQQ directly from nutrient agar slants exhibited a lag period of 16 to 24 h prior to growth. This lag period probably reflects the time necessary for the cells to acquire functional PQQ-containing glucose dehydrogenase in their periplasms. Lag periods were not detected when cells growing in glucose plus PQQ were subcultured directly into the same medium, even after two washes with 50 mM phosphate buffer. The lag period was restored if the glucose-plus-PQQ-grown cells were instead subjected to an osmotic

TABLE 1. Growth of glucose-negative *E. coli* in the presence and absence of 5% SDS

Carbon source(s)	Maximum growth (Klett units)[a] of:			
	ZSC113		DF214	
	Control	Plus SDS	Control	Plus SDS
Glucose	1	0	2	0
Glucose + PQQ	205	5	140	6
Gluconate	155	72	148	4
Glucose + gluconate	156	101	150	57

[a] Values reported are the averages of 3 to 5 experiments. Maximum growth occurred between 22 and 48 h after inoculation. Actual times were influenced by the added lag time for PQQ-dependent growth as well as the more rapid utilization of glucose by SDS-grown cells (15) and the onset of SDS-induced lysis once those energy sources had been consumed (14).

TABLE 2. Phosphate limitation and the PQQ-dependent lag period in *E. coli* ZSC113

Inoculum[a]	Glucose + PQQ growth medium[a]	Lag period (h)
Nutrient agar	High P_i	≥16
Nutrient agar	Low P_i	8
Tryptone-salts[b]	High P_i	30
Tryptone-salts	Low P_i	8
High P_i-Gluconate	High P_i	7
High P_i-Gluconate	Low P_i	5
Low P_i-Gluconate	High P_i	1

[a] High P_i = 78 mM; low P_i = 50 μM. The low P_i-gluconate medium and all glucose plus PQQ media are supplemented with 50 mM morpholinepropanesulfonic acid (MOPS) to maintain adequate buffering.
[b] Tryptone (10 g/liter) and NaCl (10 g/liter), pH 7.5.

shock (19) prior to inoculation. These results suggest a lag caused by limited or slow entry of PQQ into the periplasm and/or slow formation of functional glucose dehydrogenase from PQQ and the apoenzyme (4).

The PhoE porin. To our knowledge, the mechanism by which PQQ enters the periplasm has not been addressed. PQQ has a molecular mass of only 330 daltons, but it also has three carboxyl groups which should be negatively charged at neutral pH. A lag time of ≥16 h for PQQ-dependent growth (Table 2) suggested the possibility that PQQ uptake was limiting. Accordingly, we tested the hypothesis that PQQ entry in *E. coli* could be facilitated by the PhoE porin. The PhoE protein is induced by phosphate limitation or starvation (13), but the resulting pores are more efficient for the transport of negatively charged compounds in general (13). This idea was confirmed by the observation (Table 2) that in all cases the PQQ-dependent lag periods were reduced when cells were inoculated into low-phosphate (50 μM) medium. In particular, the lag period was only 1 h when a low-phosphate (50 μM) gluconate-grown inoculum was used.

Aerobic growth of *E. coli* DF214. *E. coli* DF214, which is able to transport and phosphorylate glucose but unable to further metabolize glucose 6-phosphate, was unable to grow on a defined glucose-containing liquid medium (Table 1). Like *E. coli* ZSC113, it grew well with either glucose plus PQQ, gluconate, or a glucose-gluconate mixture (Table 1). However, its growth pattern in the presence of 5% SDS differed from that of *E. coli* ZSC113; growth occurred only with the glucose-gluconate mixture (Table 1). The reasons for this difference are unclear. *E. coli* DF214 also exhibited a long lag period (≥20 h) for PQQ-dependent growth.

Amino acid nucleophiles. *E. coli* DF214 is a histidine auxotroph. This point is significant because van Kleef and Duine (22) showed that nucleophiles, especially amino acids, in culture media lowered the effective concentration of PQQ due to transformation of PQQ into biologically inactive compounds. The glucose-plus-PQQ-induced growth reported in Table 1 for *E. coli* DF214 (140 Klett units) was achieved with 0.1 mM histidine and 6 μM PQQ. For comparison, no growth occurred in cultures supplemented with 1.0 mM histidine and 3 μM PQQ. Further evidence that excess histidine is in fact inactivating the PQQ is provided by the observation that addition of 5 mM histidine prevents the PQQ-dependent growth of *E. coli* ZSC113 on glucose plus 3 μM PQQ. Histidine did not alter the growth of *E. coli* ZSC113 on gluconate.

Growth rate comparisons. Wild-type *E. coli* has three pathways available for the catabolism of glucose. They are the Embden-Meyerhof-Parnas, pentose phosphate, and ED pathways (10). In this regard, we compared, in minimal media, the growth rates of *E. coli* W3110 (wild type), *E. coli* ZSC113 (pentose phosphate and ED), and *E. coli* DF214 (pentose phosphate only). *E. coli* W3110 grown with glucose and ZSC113 grown with either glucose plus PQQ or gluconate exhibited generation times of ca. 65 min. In contrast, *E. coli* DF214 grown with either glucose plus PQQ or gluconate exhibited generation times of 5 to 6 h. These growth rate comparisons are made with the caveat that while strains W3110, ZSC113, and DF214 were all derived from *E. coli* K-12, they are not isogenic. The slower growth (G = 155 min) of ED-defective mutants of *E. coli* on gluconate had been observed previously by Zablotny and Fraenkel (24).

Anaerobic growth of *E. coli* ZSC113. Because animal gastrointestinal tracts are anaerobic, any assessment of the probable in situ importance of PQQ and the ED pathway must consider anaerobic conditions. However, when *E. coli* ZSC113 was incubated anaerobically in defined liquid media with either glucose plus PQQ or gluconate, the maximum growth levels were only ca. 30 Klett units. Identical growth levels were obtained in media supplemented with 1% KNO_3 or 1% sodium fumarate. For *E. coli* ZSC113, neither nitrate nor fumarate (18) can substitute for oxygen in coupling ED activity to respiration. These results are consistent with anaerobic energy production exclusively by the fermentation of gluconate (12). Isturiz et al. (12) also observed that *E. coli* grew poorly on gluconate anaerobically (G = 152 min).

DISCUSSION

We have shown that two glucose-negative mutants of *E. coli* (ZSC113 and DF214) can grow with glucose as the sole source of carbon and energy if they are also provided with the coenzyme PQQ. *E. coli* does not produce PQQ but does produce the apoenzyme of the periplasmic PQQ-dependent glucose dehydrogenase. The implications of our data are fourfold.

First, PQQ appears to have difficulty entering the periplasm of *E. coli* except under low-phosphate growth conditions. It is well documented (13) that the PhoE porin would be expressed under these conditions.

Second, would either of these glucose-negative mutants be an appropriate organism for the microbiological assay of PQQ as a required vitamin? The answer is probably no. Even though ZSC113 does exhibit PQQ-dependent growth on glucose, the problems caused by a variable, phosphate-dependent lag period, a limited range of linearity, and nucleophile inactivation of the PQQ seem too severe.

Third, like most enteric bacteria (16), *E. coli* is able to grow in the presence of high concentrations of detergents. For both *E. coli* ZSC113 and DF214, 5% SDS prevented the PQQ-dependent growth on glucose. Several mechanisms can be envisioned for this inhibition by SDS. Of these, we prefer the model (5) wherein low levels of SDS are present in the periplasmic space so that the SDS would either inactivate the glucose dehydrogenase or prevent attachment of PQQ to the apoenzyme. We cannot explain why growth of *E. coli* DF214 in SDS requires both glucose and gluconate except to note that the PTS has recently been shown (20) to exert regulatory functions as well as transport functions. In particular, the PTS regulates transcription of some operons necessary for catabolism of non-PTS sugars (20). *E. coli* DF214, unlike strain ZSC113, would be expected to transport and phosphorylate glucose. In this regard, Eisenberg and Dobrogosz (9) showed that gluconate could be cometab-

olized with glucose by cells that had been preinduced by growth on gluconate.

Finally, what is the relative importance of PQQ and the ED pathway to *E. coli* in nature? The mere fact that glucose dehydrogenase and the ED pathway have been maintained during the evolution of *E. coli* indicates their selective value in at least one environment encountered by the organism. Aerobically, the equivalent generation times of *E. coli* ZSC113 and the wild-type strain, W3110, indicate that the ED pathway can fully compensate for an inactive Embden-Meyerhof-Parnas pathway. The actual importance of the ED pathway is indicated by the ca. 5-fold slower growth rate in gluconate medium of *E. coli* DF214 (pentose phosphate only) than of ZSC113 (pentose phosphate and ED pathway). However, we have no evidence for anaerobic PQQ function by any mechanism other than fermentation of gluconate. Thus, for *E. coli*, it appears likely that the significance of PQQ and the ED pathway derives from an environment which is aerobic and phosphate limited and which does not contain many amino acid nucleophiles or detergents, that is, something other than animal gastrointestinal tracts.

This line of reasoning further predicts that *E. coli* has retained the apoenzyme for PQQ-dependent glucose dehydrogenase but not the biosynthesis of PQQ because that environment also contains a natural abundance of PQQ. In this regard, we note that some microorganisms are stimulated by the presence of PQQ even at picogram-per-milliliter levels (3), while other microorganisms, especially methylotrophs, overproduce PQQ and excrete micromolar amounts into their growth medium (2). The remaining question concerns the identity and prevalence of that PQQ-containing aquatic environment.

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