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Pyrroloquinoline Quinone, a Chemotactic Attractant for *Escherichia coli*

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***Escherichia coli* is attracted by pyrroloquinoline quinone (PQQ), and chemotaxis toward glucose is enhanced by the presence of PQQ. A *ptsI* mutant showed no chemotactic response to either glucose or PQQ alone but did show a chemotactic response to a mixture of glucose and PQQ. A strain lacking the methylated chemotaxis receptor protein Tar showed no response to PQQ.**

In *Escherichia coli*, the uptake of many sugars is catalyzed by the phosphoenolpyruvate-sugar-phosphotransferase system, PTS (15). In *Klebsiella pneumoniae*, another member of the family *Enterobacteriaceae*, glucose can also be metabolized after periplasmic oxidation by the pyrroloquinoline quinone (PQQ)-dependent glucose dehydrogenase, GLD (13). *E. coli* can synthesize the apoenzyme of GLD (3), but it is seemingly unable to synthesize PQQ (7, 8, 21). However, the enzyme can be easily reconstituted to a functional dehydrogenase by the presence of PQQ in the environment because its active center faces the periplasm (8). In view of this rather curious dependency on PQQ, it could be advantageous for *E. coli*, which is motile, to be attracted to PQQ.

In *E. coli*, there are several sensory mechanisms that operate to control motility (19). Most research has been focused on a family of methylated chemotaxis receptor proteins designated Tsr, Tar, Tap, and Trg (19, 20). Responses to oxygen (aerotaxis) depend on the presence of the electron transport system (16). Chemotaxis toward glucose depends on the PTS (11, 15) in which enzyme II acts as a receptor (2, 11, 19). Chemotaxis of *E. coli* (strains given in Table 1) toward PQQ was investigated both by using swarm plates and by the capillary assay (12). In all cases, cells were pregrown on mineral medium (MM) (per liter; 1 g of $(NH_4)_2SO_4$, 10.5 g of K_2HPO_4, 4.5 g of KH_2PO_4, 0.2 g of $MgCl_2$, 38 mg of EDTA, 15 mg of thiamine, and 0.9 mg of $FeSO_4$; carbon source, 0.5%; pH 6.8) with glycerol as the carbon source to ensure high GLD activities. *E. coli* YMC10 showed an increased chemotactic activity in the presence of 50 and 100 μM PQQ when incubated on tryptone swarm plates (1.3% tryptone, 0.7% NaCl, 0.3% agar). The swarm rate (increase in radius of the colony with time) increased with increasing concentrations of PQQ. Specifically, at 0 μM PQQ, the swarm rate was 1.79 ± 0.11 mm/h. When the concentration of PQQ was increased to 10, 50, and 100 μM, respectively, the swarm rates were 1.82 ± 0.13, 2.03 ± 0.08, and 2.75 ± 0.07 mm/h. When *E. coli* YMC10 was incubated on MM plates (MM plus 0.3% agar) with different sugars as the energy source, the presence of PQQ had a stimulating effect on the respective swarm rates (Table 2). This effect generally increased with increasing concentrations of PQQ.

More-detailed experiments were performed with the capillary assay (1, 12). Again we found that PQQ was sensed by *E. coli* YMC10. For this assay, cells were harvested in the exponential phase at an optical density at 590 nm of 0.2. After the cells were washed twice in the chemotaxis buffer (20 mM K-Pi, 1 mM $MgSO_4$, 0.1 mM EDTA), the assay was performed as described by Adler (1). The glucose concentration in all cases was 1 mM, and the PQQ concentration was as indicated in Table 2. All assays were performed in triplicate. Although the observed chemotactic activities in the absence of an energy source were low, the differences in response to different concentrations of PQQ were significant (Table 3, rows 1, 2, and 3). The addition of glucose to both the capillary and the chemotaxis chamber with the cell suspension (supplying an energy source without creating a glucose gradient) resulted in a higher response to PQQ (rows 4, 5, and 6). A concentration of 10 μM PQQ was apparently sufficient for a maximal chemotactic response. This also seemed the case when both PQQ and glucose were used as attractants (rows 7 and 8). The addition of PQQ to both the buffer and the attractant (rows 9, 10, and 11) resulted in a remarkable increase in chemotactic activity toward glucose: with 100 μM PQQ, it was about twice the activity determined in the absence of PQQ.

In a *ptsI* mutant (Lin225), a strain missing the central non-sugar-specific enzyme I and expressing high GLD activity (124 nmol of Wurster's Blue reduced · min^{-1} · mg of $protein^{-1}$ (9); protein concentrations were determined by the method of Gornall et al. [6]), chemotactic activity was restored when both glucose (0.5%) and PQQ (50 μM) were present. Without

TABLE 1. *E. coli* strains used

E. coli strain	Relevant genotype	Source and reference
AW405	ara-14 galK2 galT1 lacY1 mtl-1 xyl-5 hisG4 leuB6 thr-1(Am) *thi-1 sup tonA31*/T5 *tsx-78 rpsL136*; parental strain of AW*518*, AW539, and AW701	M. S. Springer (18)
AW518	tsr	M. S. Springer (18)
AW539	tar	M. S. Springer (18)
AW701	trg	H. Kondohl (10)
RP3525	tap	M. Slocum (17)
Lin225	ptsI	P. W. Postma (unpublished strain)
PPA297	gcd::*cam*	P. W. Postma (unpublished strain)
YMC10	endA1 thi-1 hsdR17 supE44 dlacU169 $hutC_{klebs}$	Y. M. Chen (4)

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TABLE 2. Effect of PQQ on *E. coli* YMC10 on MM plates supplemented with different sugars as the carbon source

Carbon source (0.5%)	PQQ concn (μM)	Swarm rate (mm/h)
Glucose		0.50 ± 0.01
	10	0.48 ± 0.01
	50	0.72 ± 0.01
	100	0.91 ± 0.02
Fructose		0.37 ± 0.01
	10	0.75 ± 0.04
	50	0.92 ± 0.01
	100	2.13 ± 0.01
Mannose		0.39 ± 0.01
	10	0.43 ± 0.03
	50	1.03 ± 0.02
	100	1.01 ± 0.04
Gluconate		0.37 ± 0.01
	50	1.14 ± 0.01

PQQ, no activity could be detected, whereas the addition of PQQ resulted in a swarm rate of 0.41 mm/h (± 0.03). This was not the case when fructose was used as the carbon source. The effect of PQQ on the swarm rate of the wild type toward fructose (Table 2) therefore must have been caused by a PQQ-sensing system. This was investigated using strains lacking one of the four known receptors, Tap, Tar, Trg, or Tsr and their parental strain on tryptone swarm plates with or without PQQ. From the results shown in Fig. 1, it can be concluded that PQQ was sensed by the aspartate receptor: the wild-type strain (AW405) and the strains lacking either Tsr, Trg, or Tap showed increased chemotactic activity in the presence of PQQ, whereas strain AW539 (*tar-1*) was not stimulated by the presence of this compound.

It has been shown by Galar et al. (5) that several strains of different *Rhizobium* and *Bradyrhizobium* species are attracted by PQQ. In this study, we have shown that *E. coli* shows positive chemotaxis toward PQQ (Tables 2 and 3). In this chemotaxis, the methylated chemotaxis protein Tar plays an essential role, because a mutant devoid of this protein was no longer attracted by PQQ (Fig. 1). The presence of PQQ also increased chemotaxis to glucose (Table 3).

TABLE 3. Results of the capillary assays with *E. coli* YMC10 and glucose and/or PQQ

Attractant(s)[a]	PQQ concn (μM)	Addition[b]	Activity[c]
1. PQQ	10		0.4 ± 0.7
2. PQQ	100		1.4 ± 0.7
3. PQQ	1,000		5.8 ± 0.2
4. PQQ	10	Glucose	10.2 ± 0.6
5. PQQ	100	Glucose	7.8 ± 0.5
6. PQQ	1,000	Glucose	10.6 ± 0.5
7. Glucose + PQQ	10		30.2 ± 0.2
8. Glucose + PQQ	100		28.3 ± 0.2
9. Glucose			23.3 ± 0.2
10. Glucose	50	PQQ	34.4 ± 0.4
11. Glucose	100	PQQ	47.2 ± 0.3

[a] In all cases, glucose was used in a concentration of 1 mM.

[b] The compounds mentioned in the Addition column were supplied in equal concentrations to both the capillary and the chamber.

[c] The activity is expressed as the number of bacteria that entered the capillary with the attractant (N_{at}) after 1 h compared with a capillary with 1 mM aspartate as the attractant (N_{ar}) (usual activities, 1×10^5 to 5×10^5/h), both corrected for the control with only buffer (N_b) in the capillary (usual activity, 0.3×10^4 to 1×10^4/h): $[(N_{at} - N_b)/(N_{ar} - N_b)] \times 100\%$.

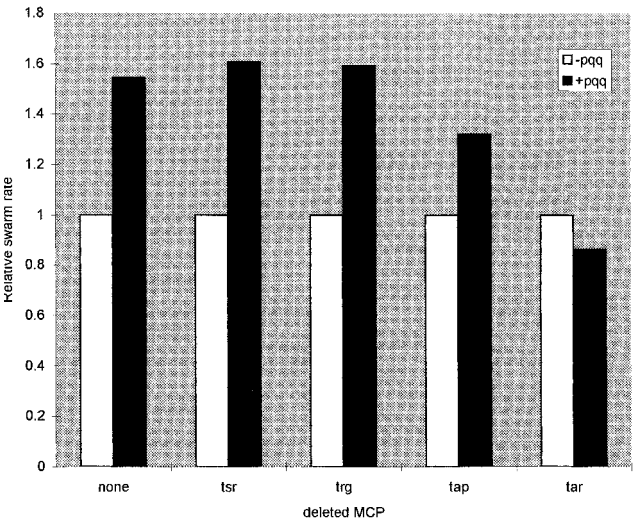

FIG. 1. Effect of PQQ on the swarm rate of *E. coli* strains lacking one of the methylated chemotaxis receptor proteins (MCPs) by using tryptone swarm plates. The swarm rate in the presence of PQQ (+pqq) of each strain was compared with the swarm rate without PQQ (−pqq), which was set at 1.

The wild-type strain YMC10 showed high GLD and PTS-glucose activities when grown on MM-glycerol (102 nmol of Wurster's Blue reduced · min^{-1} · mg of $protein^{-1}$ [9] and 40 nmol of sugar phosphorylated. min^{-1} · mg^{-1} [dry weight] [14], respectively). It has been shown previously that simultaneous degradation of glucose via the PTS and GLD in the presence of PQQ results in a faster consumption of glucose (8). Adler and Epstein (2) showed that *E. coli* cells started to swim as soon as the glucose concentration was below 100 mM and that the chemotactic activity was optimal between 1 and 10 mM (our initial glucose concentration was 28 mM). The faster consumption of glucose therefore could be responsible for the increased chemotactic response toward glucose in the presence of PQQ. It cannot be ruled out, however, that reoxidation of $PQQH_2$ or reconstitution of GLD by PQQ also played a role. The involvement of GLD in chemotaxis to PQQ could not be tested because the *gcd* mutant of *E. coli* (PPA297) that was available to us was no longer motile. But whatever role the GLD plays in the physiology of organisms such as *E. coli*, it is of substantial benefit to this organism if it can reconstitute its enzyme to a functional protein by swimming toward PQQ.

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