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NOTES

Uptake of Glycerol-2-Phosphate via the *ugp*-Encoded Transporter in *Escherichia coli* K-12[▽]

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During phenotypic characterization of various *Escherichia coli* mutants, we observed that Δ*phoA* strains are capable of using glycerol-2-phosphate (G2P) as a sole source of phosphorus. Mutations in the *ugpBAECQ* operon eliminated this phenotype, suggesting that G2P is a previously unrecognized substrate for the binding protein-dependent Ugp transporter.

Most organisms preferentially use inorganic phosphate as a source of phosphorus. Nevertheless, ecosystems are commonly limited by this essential nutrient, and living organisms frequently encounter phosphorus starvation conditions. To overcome this problem, many microorganisms have evolved systems for the acquisition of phosphorus from alternative substrates, which include a wide variety of phosphate esters, phosphonic acids and other reduced phosphorus compounds (17, 19).

There are two generic mechanisms for utilization of phosphate esters as a source of phosphorus. Many phosphate esters are hydrolyzed by extracellular phosphatases that release free phosphate, which is then transported into the cell. Other phosphate esters are transported intact and then either hydrolyzed by a cytoplasmic phosphatase or, in cases where the compound is a normal cellular metabolite, channeled directly into metabolism. The use of these alternate phosphorus sources in the enteric bacterium *Escherichia coli* has been intensively studied (for a review, see reference 17). In this organism the principal extracellular phosphatase is a nonspecific, alkaline phosphomonoesterase encoded by the *phoA* gene (4). PhoA is required for the use of a variety of phosphate esters as sole phosphorus sources; however, glycerol-3-phosphate (G3P) and G3P diesters can serve as sole phosphorus sources in the absence of PhoA after uptake by a binding protein-dependent transporter encoded by the *ugpBAECQ* operon (2, 12). The last gene in the operon, *ugpQ*, encodes a glycerol-3-phosphodiesterase that releases free G3P after transport (2). Consistent with their proposed biological roles, both *ugp* and *phoA* are tightly regulated by the availability of extracellular phosphate, along with a number of other genes involved in the use of alternative phosphorus sources. These genes, collectively known as the PHO regulon, are transcriptionally controlled by the products of the *phoBR* operons and are expressed only during phosphate starvation (15).

Gylcerol-2-phosphate (G2P), on the other hand, has been presumed to be a nontransportable substrate and, thus, dependent on PhoA for its use as a source of phosphorus. In fact, several publications have reported the use of G2P for selection for alkaline phosphatase mutants (9, 11, 14). Therefore, we were surprised to find that a Δ*phoA* strain (BW14894 [Table 1]) was able to utilize G2P (Sigma, St. Louis, MO) as a sole phosphorus source in 0.4% glucose-morpholinepropanesulfonic acid (MOPS) medium (18). We considered two possible explanations for this result.

First, it seemed possible that our G2P stock solution was contaminated by another phosphorus compound, such as G3P, that could serve as a sole phosphorus source in the absence of PhoA. To test this hypothesis, we analyzed 500 mM stock solutions of G2P and G3P (Sigma, St. Loius, MO) using ^{31}P nuclear magnetic resonance (NMR) as previously described (21). Proton-coupled ^{31}P spectra of G2P showed the expected doublet with peaks at 2.38 and 2.32 ppm, consistent with previously reported chemical shifts (3) (Fig. 1). Very small additional peaks were observed at 2.7 and 1.0 ppm, which were consistent with minor amounts (less than 1%) of G3P and

TABLE 1. Strains used in this study

Strain	Genotype	Reference
BW14893	Δ*lac*(*X74*) Δ*phoA532* Δ*phn33-30*	8
BW14894	Δ*lac*(*X74*) Δ*phn33-30*	20
WM5085	Δ*lac*(*X74*) Δ*phoA532* Δ*phn33-30* Δ*ugpBAECQ*	This study
WM5089	Δ*lac*(*X74*) Δ*phoA532* Δ*phn33-30* Δ*ugpB*	This study
WM5063	Δ*lac*(*X74*) Δ*phoA532* Δ*phn33-30* Δ*ugpC*	This study
WM5086	Δ*lac*(*X74*) Δ*phoA532* Δ*phn33-30* Δ*ugpQ*	This study
WM5087	Δ*lac*(*X74*) Δ*phoA532* Δ*phn33-30* Δ*ugpE*	This study
WM5088	Δ*lac*(*X74*) Δ*phoA532* Δ*phn33-30* Δ*ugpA*	This study
WM7184	Δ*lac*(*X74*) Δ*phoA532* Δ*phn33-30* Δ*glpT*::*aph*	This study
WM7186	Δ*lac*(*X74*) Δ*phoA532* Δ*phn33-30* Δ*glpD*::*aph*	This study

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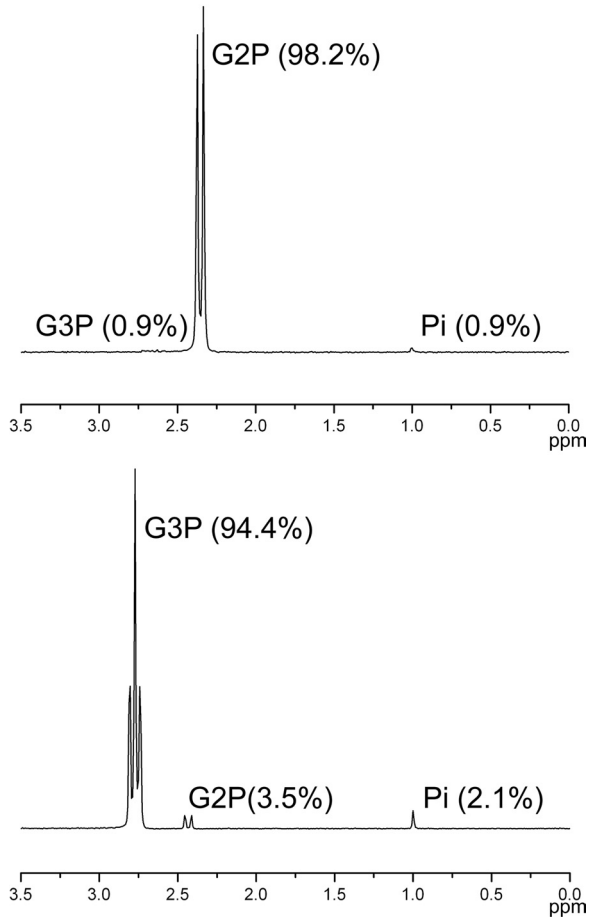

FIG. 1. Proton-coupled ^{31}P NMR spectra for G2P and G3P. Solutions (500 mM) of the highest grades commercially available were analyzed by ^{31}P NMR as described in the text. The spectrum of G2P has the predicted doublet at 2.38 and 2.32 ppm, with splitting caused by the single proton on the C-2 position. The spectrum of G3P has the predicted triplet centered at 2.80 ppm, with splitting caused by two protons at the C-3 position. Integration of the observed peaks shows that G2P is contaminated by minor amounts of phosphate (Pi) and G3P, whereas G3P has slightly higher concentrations of both phosphate and G2P. The percentage of the total phosphorus for each compound is indicated in parentheses. The spectra were aligned using the phosphate peak at 1 ppm.

phosphate, respectively, in the sample. Spectra of G3P show the predicted triplet centered at 2.8 ppm (3), accounting for 98% of the phosphorus present with 3.5% G2P and 2.1% phosphate. Control experiments conducted by spiking samples with the corresponding source at a concentration of 5% showed that additional contaminating phosphorus compounds would have been easily detected at this level; therefore, no additional phosphorus compounds were present (data not shown).

The second possibility that we considered was that our strain had a previously unrecognized *phoA*-independent pathway for assimilation of phosphorus from G2P. Because PhoA is the predominant extracellular phosphatase in *E. coli*, such a pathway would likely entail transport of G2P. Two binding protein-dependent transport systems have been shown to allow use of phosphate esters as a source of phosphorus in *E. coli*: the *ugp* system described above and the broad-specificity *phnCDE* system, which allows uptake of phosphonates, phosphate esters, and inorganic phosphate (10). *E. coli* also possesses a second G3P transporter, encoded by *glpT*, which can allow use of G3P as a phosphorus source under certain circumstances (6). The strains used in this study all have a deletion of the *phn* operon, ruling out involvement of this transport system. To test

TABLE 2. Oligonucleotide primers used for deletion construction[a]

Deletion	Direction	Primer
ugpBAECQ	Forward	**ATGAAACCGTTACATTATACAGC TTCAGCACTGGCGG**TGTAGG CTGGAGCTGCTTCG
	Reverse	**CTATTGGGCCGTAAAGTTCGGA CCAATCACGTCAA**TTTCCGGG GATCCGTCGACCTG
ugpB	Forward	**ATGAAACCGTTACATTATACAGC TTCAGCACTGGCGG**TGTAGG CTGCAGCTGCTTCG
	Reverse	**TTAAGACTTCGTCGATTTCTCAA AGCGGCGCAGCAA**TTCCGGG GATCCGTCGACCTG
ugpE	Forward	**ATGATTGAGAACCGTCCGTGGC TGACGATATTCAGCG**TGTAG GCTGGAGCTGCTTCG
	Reverse	**TTATTTCTCACTATCGACCAGGC CGCGCGCGAAGGC**TTCCGGG GATCCGTCGACCTG
ugpA	Forward	**ATGTCATCATCCCGTCCGGTGTT CCGCTCGCGCTGGG**TGTAGG CTGGAGCTGCTTCG
	Reverse	**TCATTGGTAACGCACCTTGCTTT CAACATAGCGGAA**TTCCGGG GATCCGTCGACCTG
ugpC	Forward	**ATGGCAGGACTGAAATTACAGG CAGTAACCAAAAGCG**TGTAG GCTGGAGCTGCTTCG
	Reverse	**TCATACTCGTTGTCCTGTTTCAC CATCAAAAAGATG**TTCCGGGG ATCCGTCGACCTG
ugpQ	Forward	**ATGAGTAACTGGCCTTATCCCCG CATCGTCGCTCATG**TGTAGG CTGGAGCTGCTTCG
	Reverse	**CTATTGGGCCGTAAAGTTCGGA CCAATCACGTCAA**TTCCGGG GATCCGTCGACCTG
glpT	Forward	**CATGAACAATTACTGCAAGAACG CAACGGAGGCTAATGGCGT**GT AGGCTGGAGCTGCTTC
	Reverse	**TCATCATGATCGCCATGCTAAGG TTTTTCAGCGTCAATTT**CATA TGAATATCCTCCTTAG
glpD	Forward	**CGAACATTTATGAGCTTTAACGA AAGTGAATGAGGGCAGCGT**GT AGGCTGGAGCTGCTTC
	Reverse	**CAGGCCAGATTGAAATCTGACCT GATCACCTTACGTTAAT**CATA TGAATATCCTCCTTAG

[a] Bold type indicates sequences that are homologous to regions adjacent to the genes indicated; the remainder of each primer is homologous to pKD13 or pKD4 and primes amplification of the kanamycin resistance cassette (5).

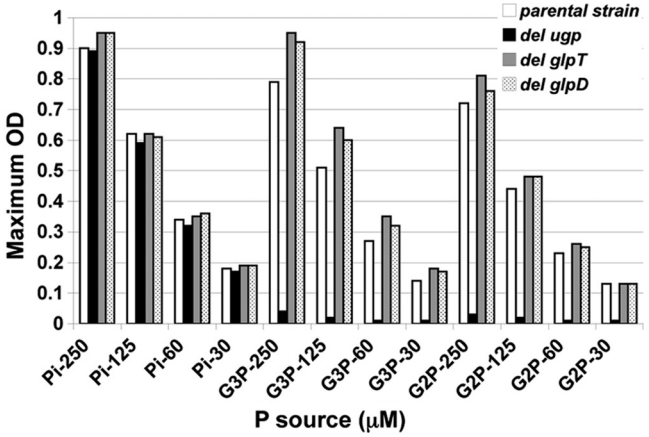

FIG. 2. Growth yields for selected *E. coli* mutants in media with G2P, G3P, and phosphate (Pi) as sole phosphorus sources. The growth yield was determined by measuring the final optical density at 600 nm (OD) after growth in 0.4% glucose-MOPS liquid media with various concentrations of the phosphorus sources. All strains are derivatives of BW14893 [Δ*lac*(*X74*) Δ*phoA532* Δ*phn33-30*]. The strains tested were BW14893 (parental strain), WM5085 (*del ugp*), WM7184 (*del glpT*), and WM7186 (*del glpD*). The concentrations used were 30, 60, 125, and 250 μM.

whether the Ugp transporter was involved, we constructed a series of deletion mutants lacking each of the *ugp* genes and lacking the entire *ugp* operon. The mutations were constructed using *E. coli* BW26678 by λ Red-mediated recombination as described previously (5), using the kanamycin resistance marker on pKD13 and the oligonucleotides shown in Table 2. Each mutation was then moved into BW14894 by P1*kc*-mediated phage transduction as described previously (16). Finally, the kanamycin resistance cassette was removed by *flp*-mediated recombination to generate nonpolar deletion mutations (5). A *glpT* mutant was constructed in a similar fashion, but the kanamycin resistance marker, which was derived from pKD4, was not removed. The final strains are shown in Table 1. The abilities of the mutants to utilize G2P, G3P, and phosphate as sole phosphorus sources were examined by scoring growth in 0.4% glucose-MOPS media with various growth-limiting concentrations of the phosphorus sources (Fig. 2). Mutants with *glpT* deletions were able to utilize both G2P and G3P as phosphorus sources, showing that *glpT* is not required for this process. In contrast, mutants lacking *ugpBEACQ* failed to grow on both G2P and G3P. Similar results were obtained with *ugpB*, *ugpE*, *ugpA*, and *ugpC* mutants (Fig. 3). Thus, in this Δ*phoA* strain background, growth on G2P and G3P requires the Ugp transporter. The phosphodiesterase-encoding gene *ugpQ* is not required for use of either compound. The observation that the growth on each substrate (in cases where growth occurred) was proportional to the level of phosphorus added and equivalent to the growth obtained with the same concentration of inorganic phosphate further corroborates the conclusion that growth was dependent on the specific phosphorus compound used and not due to contaminating compounds present in our source chemicals.

It is formally possible that G2P is enzymatically rearranged to form G3P prior to uptake by Ugp. We attempted to address this issue by introducing a mutation that would block post-transport processing of G3P. Unfortunately, the enzyme(s) that mediates assimilation of P from cytoplasmic G3P is unknown. It is known that the product of the *glpD* gene, glycerol-3-phosphate dehydrogenase, is required for use of G3P as a carbon source (13). Therefore, we constructed a *glpD* mutant (as described above for the *glpT* mutant) and tested its ability to utilize G2P and G3P as sole phosphorus sources. As shown in Fig. 2, the *glpD* mutation had no effect on the use of either phosphorus source. Thus, the possibility of conversion of G2P to G3P remains open, as does the downstream fate of G3P following transport by Ugp.

The requirement for an intact Ugp transporter for use of G2P strongly suggests that this compound is a previously unrecognized substrate for this binding protein-dependent uptake system. The Ugp system is already known to possess fairly broad specificity, since it is able to transport both G3P and a range G3P phosphodiesters (2). Further, in vitro studies with the single-subunit GlpT transporter have indicated that it can transport both G2P and G3P, providing a precedent for recognition of both molecules by a G3P uptake system (1). In contrast to G3P, which is a major component of phospholipids, G2P is not commonly thought of as a natural product. Nevertheless, recent studies have shown that G2P is produced by certain organisms (7) and hence

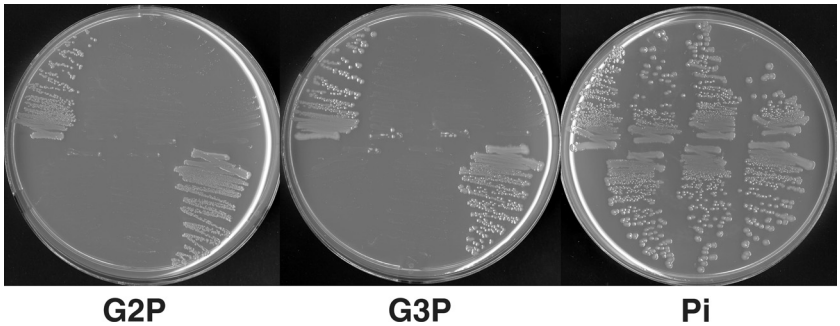

FIG. 3. Utilization of G2P, G3P, and phosphate (Pi) as sole phosphorus sources by individual *ugp* mutants. Strains were streaked on 0.4% glucose-MOPS media with the phosphorus sources indicated at a concentration of 0.5 mM. All strains are derivatives of BW14893 [Δ*lac*(*X74*) Δ*phoA532* Δ*phn33-30*]. The following strains were tested (from left to right): top row, BW14893, WM5085 (Δ*ugpBAECQ*), WM5089 (Δ*ugpB*), and WM5087 (Δ*ugpE*); bottom row, WM5088 (Δ*ugpA*), WM5063 (Δ*ugpC*), and WM5086 (Δ*ugpQ*).

should be considered an available phosphorus source in nature. Thus, a plausible selective pressure for evolution of G2P transport can also be envisioned. Further studies are required to determine the intracellular pathways for assimilation of phosphorus from both G2P and G3P.

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