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P_i Exchange Mediated by the GlpT-Dependent *sn*-Glycerol-3-Phosphate Transport System in *Escherichia coli*

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The GlpT system for *sn*-glycerol-3-phosphate transport in *Escherichia coli* is shown to catalyze a rapid efflux of P_i from the internal phosphate pools in response to externally added P_i or glycerol-3-phosphate. A *glpR* mutation, which results in constitutive expression of the GlpT system, is responsible for this rapid P_i efflux and the arsenate sensitivity of several laboratory strains, including the popular strain C600. Glucose and other phosphotransferase system sugars inhibit P_i efflux by repressing *glpT* expression.

Wild-type strains of *Escherichia coli* contain two major systems for the uptake of P_i. One of these, the phosphate-specific transport (Pst) system is characterized as a binding protein system (19, 22) which is part of the *pho* regulon (21). The other is the phosphate (inorganic) transport (Pit) system (13, 18, 22), which is energized by the proton motive force (14). It was proposed by Rosenberg et al. (15) that, in addition to its role in the active uptake of P_i, the Pit system was also involved in mediating a rapid exchange between the internal (ester plus inorganic) phosphate pools and P_i in the external medium.

The present study aims to clarify the link between the Pit system, P_i exchange, and arsenate sensitivity. In the course of this work, we discovered that some laboratory strains contained a *glpR* mutation, which maps near the *pit* gene at ca. 77 min on the *E. coli* chromosome and that *glpR* mutants exchange P_i and cause arsenate sensitivity because they express the *glpT*-dependent *sn*-glycerol-3-phosphate (*sn*-G3P) transport system constitutively.

MATERIALS AND METHODS

Bacterial strains. All strains (Table 1) were derivatives of *E. coli* K-12. P1 transduction was carried out according to the procedure of Miller (11).

Materials. The chemicals used were of the highest purity available commercially. Carrier-free $^{32}P_i$ (20 mCi/ml) and L-[*U*-^{14}C]G3P (170 mCi/mmol) were purchased from the Radiochemical Centre, Amersham, Buckinghamshire, United Kingdom. 3-(*N*-morpholino)-2-hydroxy-propanesulphonic acid (MOPSO) was prepared from 3-chloro-2-hydroxy-propanesulphonate by a modification (N. Good, personal communication) of the procedure described by Ferguson et al. (5). The purity of the recrystallized product was confirmed by elemental analysis. Phosphonomycin was a gift from Charles W. Mushett of Merck, Sharp & Dohme, Rahway, N.J.

Media. Cells were grown overnight with shaking at 37°C in the 56 minimal medium with supplements described previously (12, 14). Phosphate-free buffered "uptake" medium (pH 6.5; 37°C) contained 25 mM MOPSO, 40 mM KCl, 100 mM $(NH_4)_2SO_4$, and 1 mM $MgSO_4$. Cells were starved of P_i in uptake medium, containing the appropriate carbon source, as described previously (13).

Methods. A *glpT* mutant of strain HR159 was selected by spreading a lawn of cells onto a minimal lactate plate into which a center well was cut and filled with 50 μl of 0.2 M phosphonomycin (Fos). After 2 days of incubation at 37°C, a zone of growth inhibition 5 cm in diameter in which several hundred Fos^r colonies were growing was apparent. Several of these were picked and purified by single-colony isolation and tested for growth on minimal agar plates containing glucose, mannitol, lactate, or α-glycerophosphate as the sole carbon source. One colony, which grew on all but the α-glycerophosphate plate, was designated strain HR170.

Uptake of 0.1 mM [*U*-^{14}C]G3P was measured in uptake medium containing the appropriate energy source at 20 mM by using the techniques and apparatus described elsewhere (15). $^{32}P_i$ uptake and exchange was measured as described previously (13, 15). LB medium (11) was used for growth of cells in P1 transduction. Tetracycline-resistant colonies (Tc^r) were selected on LB medium containing 20 μg of tetracycline per ml. For the preparation of plates media were solidified with 1.5% (wt/vol) agar (Difco Laboratories, Detroit, Mich.). Arsenate-resistant (Asi^r) colonies were screened on LB plates containing 20 mM arsenate and 1 mM P_i. G3P auxotrophs were screened on low-P_i plates which contained 20 mM glucose in uptake medium with 0.5 mM P_i as the sole phosphorus source.

RESULTS

Transductional mapping of the *pit* region was done by using the *zhg*::Tn*10* strain, JGC127, as donor and strain K10 as recipient. Tetracycline-resistant transducants were tested for transfer of the nearby *pit* locus by screening for arsenate sensitivity and P_i exchange. During the course of this study it became apparent that the arsenate sensitivity and phosphate exchange were not always associated. Instead, two loci nearby the *zhg* marker could determine arsenate sensitivity: the wild-type *pit*$^+$ allele or the mutant *glpR* allele. Only the latter mutant allele caused a phosphate exchange. Also, a *pit*$^+$ allele is required for growth on P_i as a phosphorus source in a mutant lacking the Pst system, as described previously (18).

Table 2 contains the results of P1 transductions with strain JGC127 (*zhg*::Tn*10*) as donor and strains K10 (*pit*) or AN259, known from previous experiments to exchange P_i (13, 15), as recipients. The construction of strains used in experiments described elsewhere in the paper is also shown in Table 2.

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TABLE 1. *E. coli* strains used

Strain	Genotype	Source (reference)
K10	*pit*	CGSC[a] via B. Bachmann
AN259	*argH entA thi glpR*	(13)
AN710	*argH entA thi glpR phoT101*	(13)
AT2535	*thi pyrB his argH purC rpsL xyl mtl*	J. Pittard via F. Gibson
DG17	*thi malA argG metB his leu thyA mtl xyl-7 gal lacY rpsL tonA tsx* λ^r λ^- *supE*	A. Kornberg via N. Dixon
HR69	*zhg*::Tn*10*	P1 JGC127 → K10; Tc^r; exchange negative
HR92	*argH entA thi glpR zhg*::Tn*10*	P1 JGC127 → AN259; Tc^r; exchange positive
HR41	*pit zhg*::Tn*10*	P1 HR92 → K10; Tc^r; exchange negative
HR159	*pit zhg*::Tn*10 glpR*	P1 HR92 → K10; Tc^r; exchange positive
HR134	*thi pyrB his argH purC rpsL xyl mtl*	P1 HR92 → AT2535; Tc^r; exchange negative
HR136	*thi purB his argH purC rpsL xyl mtl glpR*	P1 HR92 → AT2535; Tc^r; exchange positive
HR170	*pit zhg*::Tn*10 glpR glpT*	Fos^r derivative of HR159
GS5	F^- *pit-1 pst-2 proC purE thyA nal metB his pyrF*	(18)
C600	*thi thr leu glpR*	CGSC via B. Bachmann
AC109	*ilv ugpA704*::Tn*10 glpR phoA8 glpK*	W. Boos
JGC127	*argG metB his leu thyA mtl xyl malA1 gal lacY rpsL tonA tsx* λ^r λ^- *supE*	(7)

[a] CGSC, Coli Genetic Stock Center, Yale University, New Haven, Conn.

Table 2 shows that when strain K10 was used as recipient and transduced to Tc^r with strain JGC127 as donor, 8% of the Tc^r colonies were arsenate sensitive but none of these exchanged P_i. When strain AN259 was transduced to Tc^r with a P1 lysate from strain JGC127, Tc^r transductants were obtained which either did or did not exchange P_i. One transductant, strain HR92, which showed a P_i exchange of greater than 75%, was then used as a donor to transduce strain K10 to Tc^r, and 30% of the Tc^r transductants obtained were very sensitive to arsenate and also exchanged P_i. The wild-type *pit*$^+$ locus, however, was only 5% linked to the *zhg*::Tn*10* locus. This was shown by using strains HR69 or HR92 (described in Table 2) as donors to transduce the *pit pst* strain GS5 to Tc^r and screening the transductants for growth on minimal glucose plates containing 0.5 mM P_i as the sole phosphorus source.

TABLE 3. Location of the gene responsible for P_i exchange by P1 transduction[a]

Phenotype scored:		No. of transductants in each class
Mal	P_i exchange	
+	−	1
−	+	5
−	−	54
+	+	35

[a] The recipient was DG17 (*malA* $Exch^-$). The donor was HR92 (*zhg*::Tn*10* $Exch^+$). Selection was for Tc^r. The Tc^r transductants were purified by single-colony isolation, and a lactate-grown culture of each organism was tested for [*U*-^{14}C]G3P uptake and $^{32}P_i$ uptake and exchange by the methods described previously.

The position of the gene conferring P_i exchange was ordered relative to *malA* by carrying out the three-factor cross described in Table 3 (Davis et al. [4] showed that the *zhg*::Tn*10* locus was 40% linked to *malA*). These data showed that the gene responsible for P_i exchange must lie very near *malA* but between that locus and the *zhg*::Tn*10* transposon. Figure 2 shows the linkage of *zhg*::Tn*10* to *glpR* and other loci in the 76 to 77-min region of the *E. coli* genetic map.

This P1 mapping data, taken together with the published observations that the GlpT-dependent uptake system can transport both P_i and arsenate (9, 22), strongly suggested that the gene conferring P_i exchange may be a *glpR* mutation, which would result in constitutive expression of the GlpT system (6). When an isogenic pair of strains which were either P_i exchange positive (HR136) or P_i exchange negative (HR134) (see Table 2 for derivation) was tested for uptake of 0.1 mM [U-^{14}C]G3P, it was found (Fig. 1) that the exchange-positive strain, HR136, showed a constitutive level of G3P uptake, whereas the exchange-negative strain, HR134, showed a level of G3P uptake expected for cells not induced for the GlpT system. The correlation between constitutive uptake of G3P (*glpR*) and P_i exchange is confirmed by the P1 mapping data presented in Table 3. In that experiment, all transductants which exchanged P_i also constitutively expressed GlpT transport activity.

The arsenate sensitivity and phosphate exchange-positive phenotypes of strain HR159 (see Table 2) apparently are due to the *glpR* mutation and not to a wild-type *pit*$^+$ allele. The presence of the mutant *pit* allele in strain HR159 was demonstrated by using it as a transductional donor with strain AN710 as recipient. The latter strain is mutated in the Pst system and, as a consequence, is constitutive for alkaline phosphatase. It can still use P_i as a source of phosphorus

TABLE 2. Transductional analysis of loci, linked to *zhg*::Tn*10*, responsible for arsenate sensitivity and P_i exchange (selection for Tc^r)

Strain (relevant genotype or phenotype)		Recombinant class (phenotype)	% Of total	Derived strain designation
Donor	Recipient			
JGC127 (*zhg*::Tn*10* Asi^s $Exch^-$)[a]	K10 *pit* Asi^r $Exch^-$)	Asi^s $Exch^-$	8 (3/36)	HR69
		Asi^r $Exch^-$	92 (33/36)	HR41
JGC127 (*zhg*::Tn*10* Asi^s $Exch^-$)	AN259 (Asi^s $Exch^+$)	$Exch^+$ Asi^s	61 (95/155)	HR92
		$Exch^-$ Asi^s	39 (60/155)	
HR92 (*zhg*::Tn*10* Asi^s $Exch^+$)	K10 (*pit* Asi^s $Exch^-$)	$Exch^+$ Asi^s	30 (29/96)	HR159
		$Exch^-$ Asi^s	70 (67/96)	
HR92 (*zhg*::Tn*10* Asi^s $Exch^-$)	AT2535 (Asi^s $Exch^-$)	$Exch^+$ Asi^s	40 (4/10)	HR136
		$Exch^-$ Asi^s	60 (6/10)	HR134
HR69 (*zhg*::Tn*10* Asi^s $Exch^-$)	GS5 (*pit pst* Pi^-)	Pi^+ *pit*$^+$	5 (5/100)	
HR92 (*zhg*::Tn*10* Asi^s $Exch^+$)	GS5 (*pit pst* Pi^-)	Pi^+ *pit*$^+$	8 (8/100)	

[a] Asi^s, Sensitivity to 10 mM arsenate; Asi^r, resistance to 10 mM arsenate (see the text); $Exch^+$, exchange-positive phenotype; $exch^-$, failure to exchange P_i.

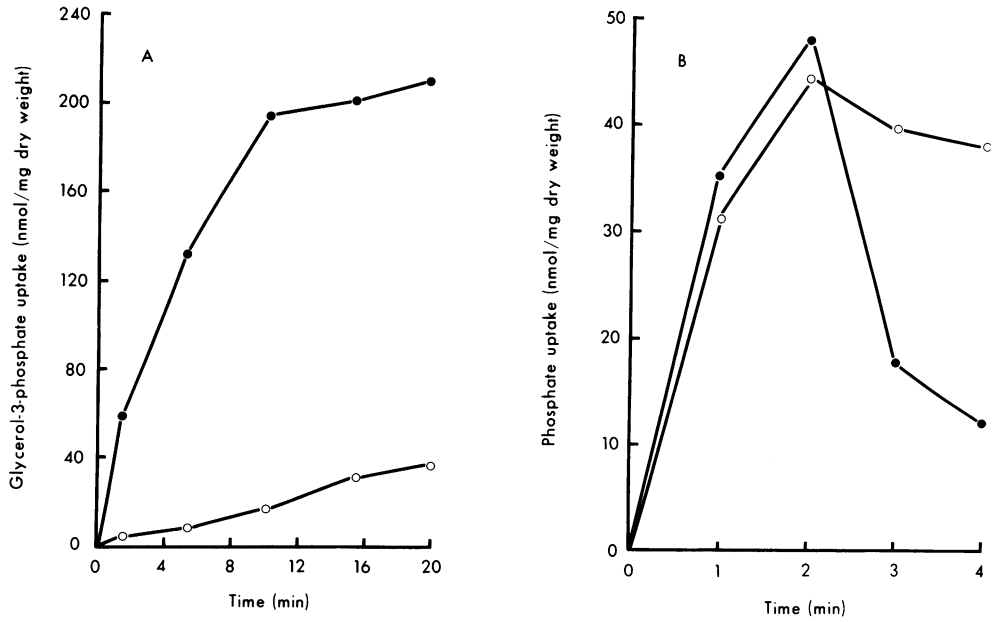

FIG. 1. Uptake of [*U*-^{14}C]G3P (A) and uptake and exchange of $^{32}P_i$ (B) by strains HR134 (*glpR*$^+$) and HR136 (*glpR*). Cells were grown aerobically at 37°C overnight in 56 minimal medium containing 20 mM DL-lactate as carbon source. For measurement of [U-^{14}C]G3P uptake (A), the cells were grown to mid-exponential phase in the medium used for overnight growth, washed twice, and then resuspended to a density of 0.15 mg (dry weight)/ml in P_i-free buffer containing 20 mM DL-lactate. The uptake of 0.1 mM [*U*-^{14}C]G3P (A) was measured as described in the text. $^{32}P_i$ uptake and exchange (B) was measured as described previously (15), with 20 mM unlabeled P_i added at $t = 2$ min. Open circles, Strain HR134; closed circles, strain HR136.

because it has a functional Pit system. Recombinants which are defective in both the Pst and Pit systems, however, are P_i auxotrophs. Since 4% of the Tc^r transductants of strain AN710 became auxotrophic for G3P, we conclude that strain HR159 has a mutant *pit* allele, as expected.

Stimulation of P_i efflux by G3P. Since exchange-positive strains were shown to express the GlpT system constitutively, it was of interest to determine whether the physiological substrate of that transport system, *sn*-G3P, could itself stimulate efflux of P_i. Strains HR159 and HR41 were tested for the effect of various concentrations of unlabeled G3P on $^{32}P_i$ efflux. We found (Table 4) that although at least 10 mM unlabeled P_i was required to cause maximal efflux of $^{32}P_i$ in strain HR159, G3P stimulated the same rate and extent of $^{32}P_i$ efflux from those cells at only 0.1 mM. In contrast, neither G3P (even at 10 mM) nor 10 mM P_i stimulated significant $^{32}P_i$ efflux from lactate- or succinate-grown cells of strain HR41, which would be uninduced for the GlpT system. Arsenate and phosphonomycin showed a pattern similar to P_i, being effective in strain HR159 but not in HR41. In the absence of an added substrate of the G3P transporter, $^{32}P_i$ accumulated by P_i-starved cells remained inside the cells.

***glpT* mutants fail to exchange P_i.** A phosphonomycin-resistant mutant of strain HR159 was selected (see above) and shown to carry a *glpT* mutation (20). This *glpT* mutant, strain HR170, failed to take up 0.1 mM [*U*-^{14}C]G3P or to exchange $^{32}P_i$ for either $^{31}P_i$ or G3P. Its rate of $^{32}P_i$ uptake

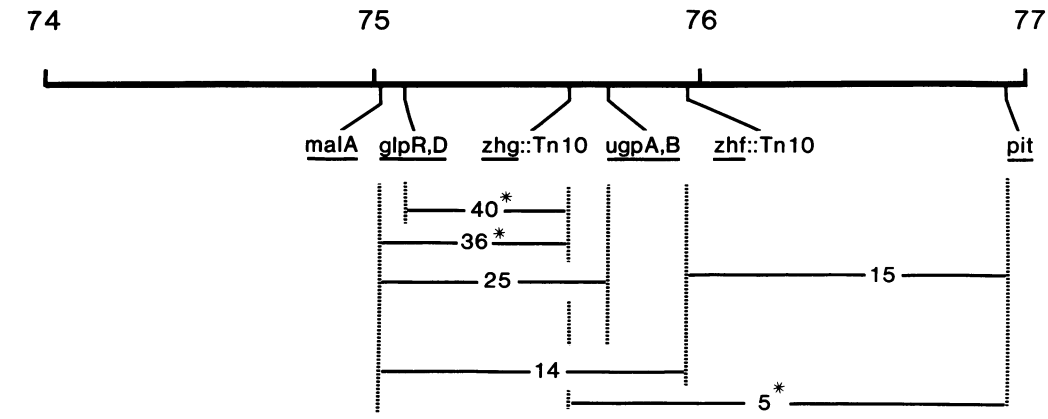

FIG. 2. Linkage of *zhg*::Tn*10* to the *glpR*, *pit*, and *malA* loci in the 75- to 77-min region of the *E. coli* genetic map. Positions of the indicated markers in the 75- to 77-min region of the chromosome are redrawn from Bachmann (2) and Schweizer et al. (17). The numbers below are cotransduction frequencies; those with asterisks are our data; the remaining loci and cotransduction frequencies are taken from reference 17.

TABLE 4. Effect of various GlpT substrates on $^{32}P_i$ efflux from cell suspensions of strain HR41 or HR159[a]

Strain (relevant genotype) and substrate added	% Exchange at concn of substrate (mM):		
	0.1	1.0	10.0
HR159 (*glpR*)			
G3P	73	81	83
P_i	14	39	70
Arsenate	38	70	74
Phosphonomycin	24	28	35
HR41 (*glpR*$^+$)			
G3P	−6[b]	−9	−7
P_i	8	9	12
Arsenate	5	8	14
Phosphonomycin	−11	−16	−12

[a] Cells were grown overnight at 37°C in 56 minimal medium containing 20 mM K^+ DL-lactate as the sole carbon source. The cultures were washed in P_i-free medium containing 20 mM lactate and starved of P_i at 37°C for 2 h as described (15). The cells were then resuspended to a density of 0.15 mg/ml in the same P_i-free medium. $^{32}P_i$ (6 μCi/ml) added to 50 μM at zero time. At *t*-2 min unlabeled G3P, P_i, arsenate, or phosphonomycin was added at the concentrations shown. The data represent the ^{32}P label lost at *t*-4 min as a percentage of $^{32}P_i$ accumulated at *t*-2 min.

[b] A negative number means that uptake continued after the addition.

was normal since it carries a wild-type Pst system. These data confirm the involvement of the GlpT-dependent G3P transport system in mediating both P_i-P_i exchange and G3P:P_i exchange.

GlpT transport system is a coupled G3P-P_i antiporter. We suspected that the rapid P_i efflux mediated by the GlpT system may be obligatorily coupled to the high inward flux of G3P. To show this dependence, it was necessary to deplete the internal ester-plus-P_i pools by starving cells of P_i. Under these conditions, however, alkaline phosphatase, the Ugp transport system, and the Pst transport system, which are all under *pho* regulon control (16, 21), would be derepressed. The [U-^{14}C]G3P could be cleaved by the periplasmic alkaline phosphatase, thereby liberating P_i, which could enter the cells via the high-affinity Pst transport system. Alternatively, G3P could be taken up intact via the Ugp transport system (16, 17). When these systems were eliminated, either by inhibiting their synthesis with chloramphenicol during P_i starvation (data not shown) or by using the mutant strain AC109 (*ugpA phoA*) (kindly provided by W. Boos), the requirement for P_i efflux with G3P uptake by the GlpT system became apparent (Fig. 3). Prior addition of 50 μM unlabeled P_i caused a six- to sevenfold stimulation in the rate of 0.1 mM [U-^{14}C]G3P uptake.

DISCUSSION

The present work shows that phosphate exchange described earlier in certain strains of *E. coli* and attributed to the Pit system (15) is due actually to the constitutive activity of the GlpT-dependent *sn*-G3P transport system. Since this rapid P_i efflux is seen in *glpR* strains that are devoid of either the Pit or Pst systems, we conclude, as was suggested by Willsky and Malamy (24), that both major P_i transport systems in *E. coli* are essentially unidirectional.

We also show that $^{32}P_i$ accumulated via the Pit or Pst system in such *glpR* strains fails to exit from those cells unless P_i or G3P, the substrate of the GlpT transport system, is added to the external medium. The concentration of G3P required to elicit this response is at least two orders of magnitude lower than that for P_i, arsenate, or phosphonomycin, which are known to be substrates of the GlpT carrier (3, 8, 18, 20, 22). Phosphonomycin-resistant mutants, which lack the GlpT system (20), fail to exchange internal P_i for either external P_i or G3P.

It is thus apparent that the GlpT transport protein does not merely facilitate P_i exit, but rather that the inward movement of a substrate on that carrier is linked to the outward expulsion of P_i, as predicted by Schweizer et al. (16). Since the GlpT system is pmf driven, yet transports G3P even at pH 7.5, electrogenic entry involving the concomitant movement of three protons with each G3P has to be postulated. The obligatory counterflow of P_i as a means of energy coupling is a more attractive proposition (W. Boos, personal communication). In this respect, we wish to highlight the similarities of this anion antiport mechanism in *E. coli* with the novel anion-antiport system recently discovered in *Streptococcus lactis* (1, 10) involving a hexose-phosphate transport system.

In two previous reports from this laboratory (13, 15) it was noted that P_i exchange was absent in cells grown on glucose or other phosphotransferase sugars. We now interpret these observations to be the result of the known catabolite-sensitive nature of *glpT* expression (6).

Previous observations have also suggested arsenate sensitivity as a useful screen for the presence of the Pit system

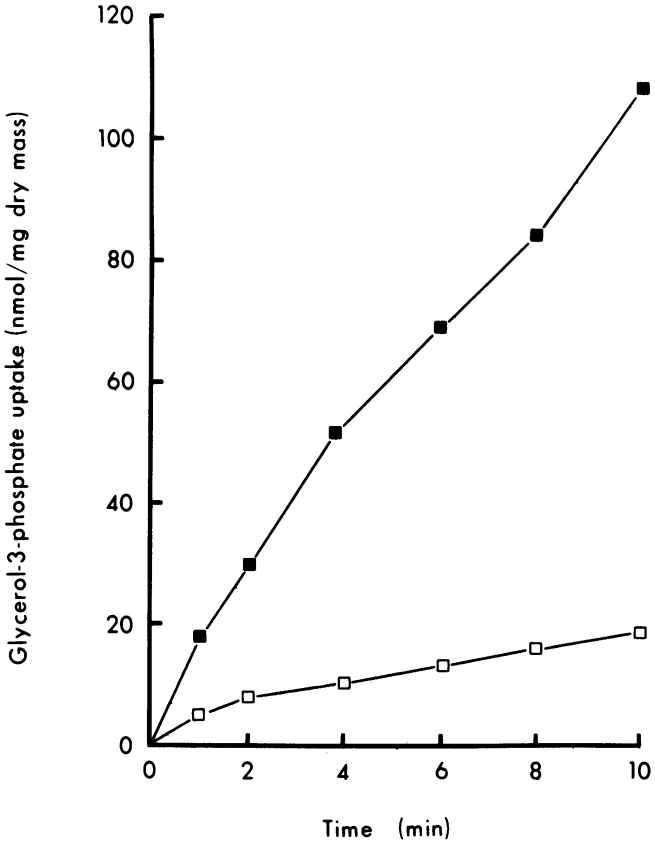

FIG. 3. Stimulation of [*U*-^{14}C]G3P uptake by P_i in strain AC109 (*ugpA phoA*). Cells were grown aerobically at 37°C overnight in 56 minimal medium containing 20 mM DL-lactate as carbon source and P_i starved as described in the text. The cells were resuspended in P_i-free uptake medium at a density of 0.15 mg (dry weight)/ml, and the uptake of 0.1 mM [*U*-^{14}C]G3P was measured in the absence (open squares) or presence (closed squares) of 50 μM unlabeled P_i which was added 2 min before the [U-^{14}C]G3P.

(13, 15, 23–25). In the course of our studies, we noted that *glpR* mutants were consistently more sensitive to arsenate than were Pit^+ strains which were inducible ($glpR^+$) for the GlpT system. Thus, even though a mutant *pit* allele causes an arsenate resistance phenotype, it will not do so in a background that is $glpR\ glpT^+$.

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