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A Second Transport System for *sn*-Glycerol-3-Phosphate in *Escherichia coli*

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Strains containing phage Mu*cts* inserted into *glpT* were isolated as fosfomycin-resistant clones. These mutants did not transport *sn*-glycerol-3-phosphate, and they lacked GLPT, a protein previously shown to be a product of the *glpT* operon. By plating these mutants on *sn*-glycerol-3-phosphate at 43°C, we isolated revertants that regained the capacity to grow on G3P. Most of these revertants did not map in *glpT* and did not regain GLPT. These revertants exhibited a highly efficient uptake system for *sn*-glycerol-3-phosphate within an apparent K_m of 5 μM. In addition, three new proteins (GP 1, 2, and 3) appeared in the periplasm of these revertants. None of these proteins were antigentically related to GLPT. However, like GLPT, GP 1 exhibits abnormal behavior on sodium dodecyl sulfate-polyacrylamide gels. GP 2 is an efficient binding protein. The new uptake system showed different characteristics than the system that is coded for by the *glpT* operon. It was inhibited neither by phosphate nor fosfomycin. So far, none of the systems that transport organic acids in *Escherichia coli* could be implicated in the new *sn*-glycerol-3-phosphate uptake activity. The mutation *ugp*$^+$, which was responsible for the appearance of the new transport system and the appearance of GP 1, 2, and 3 in the periplasm was cotransducible with *araD* by phage P1 transduction and was recessive in merodiploids.

sn-glycerol-3-phosphate (G3P) is normally transported in *Escherichia coli* by only one system that is coded for by the *glpT* operon. This is clear from the observation that mutations in *glpT* abolish the uptake of G3P (12, 18). The *glpT* operon is regulated by the product of *glpR*, the repressor of the *glp* regulon, with G3P as the inducer (6). In addition, it is subjected to catabolite repression and respiratory control (8). The *glpT* operon is positioned at 48 min on the *E. coli* chromosome (6, 15, 29), 50% cotransducible with *nalA*. The transport activity is characterized by a K_m of 12 μM for G3P uptake, whereas inorganic phosphate and fosfomycin can also be transported with K_m values in the millimolar range (12).

Recently, we found a periplasmic protein, GLPT, that is coded for by a gene within the *glpT* operon (1, 29). However, the function of this protein for G3P transport remains unclear (2). To establish whether or not GLPT plays an essential role in the mechanism of the G3P transport system, we attempted to isolate mutants that would result in a structurally altered GLPT protein. If GLPT participates in the transport mechanism, such an alteration would probably alter transport activity. Along these lines, we planned to isolate revertants that would arise from insertions of phage Mu*cts* in *glpT* by selecting for growth on G3P at 43°C. By an incorrect excision of phage Mu, one would then expect strains that synthesize an altered GLPT protein. Such an alteration might then be recognized by charge or molecular-weight differences in comparison to the wild-type GLPT protein.

Revertants that arose by this selection procedure are described in this paper.

MATERIALS AND METHODS

The bacterial strains, all of which are derivatives of *E. coli* K-12, that were used, constructed or isolated are listed in Table 1.

The *glpT*::Mu*cts* strains were isolated by plating Mu*cts* lysogens (28) on tryptone yeast extract agar (21) containing fosfomycin (Merck & Co., Inc.) as described by Venkateswaran and Wu (30). Resistance to fosfomycin can be the result of a variety of mutations. To maximize the number of *glpT* insertions so obtained, we started with strain TS100, which expresses the *glp* regulon constitutively (*glpR*).

All genetic manipulations, such as phage P1 transduction, episome transfer, Hfr crosses, and selection for nalidixic acid resistance (*nalA*), were done as described by Miller (21). To score for growth on G3P, cells were patched on low-phosphate-medium (9) agar

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TABLE 1. *Bacterial strains (E. coli K-12 derivatives)*

Strain no.	Parent	Method of isolation	Known markers	Known relevant phenotype	Source/reference
LA3400			Hfr *phoA*	GLPT$^+$ (GP1, 2, 3)$^-$	29
LA3430	CSH74	Resistance to fosfomycin, spontaneous	Hfr *glpT*	GLPT$^-$ (GP1, 2, 3)$^-$	
LA3431	LA3430	Resistance to nalidixic acid, spontaneous	Hfr *glpT nalA*	GLPT$^-$	This study
LA108	BUG-6	Growth on β-glycerol-phosphate	F$^-$ *gal xyl mtl rpsL phoC ugp*$^+$[a]	GLPT$^+$ (GP1, 2, 3)$^+$, temperature sensitive for cell division	23, 27
LA3432	LA3430 × LA108	Hfr cross selection *rpsL, gal*	F$^-$ *rpsL nalA glpT ugp*$^+$[a]	GLPT$^-$ (GP1, 2, 3)$^+$, temperature sensitive for cell division	This study
LA3433	LA3430 × LA108	Hfr cross selection *rpsL, gal*	F$^-$ *rpsL nalA glpT*	GLPT$^-$ (GP1, 2, 3)$^-$, temperature sensitive for cell division	This study
LA3427	LA5301	P1 lysate of LA3400 transduced into LA5301 selecting on arabinose, streptomycin	F$^-$ *glpT ara*$^+$ *ugp*$^+$	GLPT$^-$ (GP1, 2, 3)$^+$	This study
LA3428	LA5301	P1 lysate of LA3400 transduced into LA5301 selecting on arabinose, streptomycin	F$^-$ *glpT ara*$^+$	GLPT$^-$ (GP1, 2, 3)$^-$	This study
LA3429	JK114R3	Resistance to nalidixic acid, spontaneous	F$^-$ *argH ara lac gal ura trp his thi mal man xyl purc tonA rpsL cir nalA mgl*	GLPT$^+$	5
EB102			Δ*lac* Δ(*ara-leu*)*498* F′ *thr araC* (KLF1)		
EB103			Δ*lac* Δ(*ara-leu*)*498* F′ *thr araB* (KLF1)		Obtained from E. G. Bade
EB104			Δ*lac* Δ(*ara-leu*)*498* F′ *thr araA* (KLF1)		
TS100	MC4100		F$^-$ *araD139* Δ*lacU169 thi relA rpsL glpR*	GLPT$^+$ (GP1, 2, 3)$^-$	28
TS101, 2, 3, 4, 6, 7, 8, 9, 10, 12, 13, 18, 20, 24, 26, 29, 31, 32, 34, 37	TS100	Mu*cts* lysogenization and subsequent selection on fosfomycin	*glpT*::Mu*cts* TS104 is *glpT* and contains Mu*cts* elsewhere in the chromosome	All but TS104 lack GLPT	This study
LA5000	TS100	P1 lysate of LA3429 transduced into TS100, 1, 2, 3 etc. selecting for nalidixic acid resistance	*glpT*$^+$ *ugp*$^+$[a] *nalA*	GLPT$^+$, small amounts of GP1, 2, 3	This study
LA5001, 2, 3 etc.	TS101, 2, 3 etc.	P1 lysate of LA3429 transduced into TS100, 1, 2, 3 etc. selecting for nalidixic acid resistance	*glpT*::Mu*cts nalA* LA5004 is *glpT nalA* and contains Mu*cts* elsewhere in the chromosome	All but LA5004 lack GLPT; all contain Mu*cts*; none contains GP1, 2, 3	This study
LA5101					
LA5201	LA5001				
LA5301					
LA5124	LA5024	Growth on G3P at 43°C	*glpT*::Mu *nalA ugp*$^+$[b]	GLPT$^-$ (GP1, 2, 3)$^+$, lysogenic for Mu	This study
5224					
LA5137					
5237	LA5037				
5337					
LA5129	LA5029				
LA5107	LA5007		*glpT*$^+$ Mu *nalA*	GLPT$^+$ (GP1, 2, 3)$^-$, lysogenic for Mu	
LA5109	LA5009				
LA5104	LA5004		*glpT ugp*$^+$ Mu *nalA*	GLPT$^+$ (GP1, 2, 3)$^+$, lysogenic for Mu	
LA5204	LA5004		*glpT*$^+$ Mu *nalA*	GLPT$^+$ (GP1, 2, 3)$^-$, lysogenic for Mu	

[a] The genotype *ugp*$^+$ in these strains has not been confirmed by transduction.
[b] In these mutants, the genotype *ugp*$^+$ has only been determined by transduction in strain LA5301 and 5337.

plates containing 0.2% D,L-α-glycerol-phosphate.

To check for the presence of lysogenic Mu, cells were replica plated on LB plates (21) and overlaid with H-top agar (21) containing a phage Mu-sensitive indicator strain. Plaque formation indicated the presence of Mu lysogens.

To check for phage Mu resistance, the strains were streaked on DYT plates, and a droplet of a lysate of Mu*cts*62, *mom* (for modification of Mu) was added. The plates were screened for lysis after 5 h of incubation at 35°C.

To isolate the periplasmic shock fluid, the cells were grown overnight at 30°C in 500 ml of DYT medium (21) containing 0.2% glycerol. Osmotic shock was done by the method of Neu and Heppel (22), and further treatment was as described previously (29). Fluid containing about 10 mg of total shock protein was obtained routinely from a 500-ml culture.

Analytical techniques. Two-dimensional polyacrylamide gel electrophoresis of periplasmic proteins was performed as described (14) with further modification (29); 12.5% polyacrylamide slab gel electrophoresis was done by the method of Laemmli (16). Electrophoresis buffer consisted of 0.25 M tris-(hydroxymethyl)aminomethane (Tris), 0.19 M glycine (pH 8.3), and 0.1% sodium dodecyl sulfate (SDS). To demonstrate the characteristic behavior of GLPT in SDS, protein samples were applied on the gel after the addition of 1% SDS–1% dithiothreitol with or without heating at 100°C for 10 min. Electrophoresis was performed for 16 h at 75 V and at a final amperage of 5 mA. Gels were stained with Coomassie brilliant blue for 2 h and destained overnight.

Analytical electrofocusing of periplasmic proteins was done on ampholite PAG plates (pH 3.5 to 9; LKB), according to the procedure recommended by the manufacturer. The purification and characteristics of the new periplasmic proteins GP 1 and 2 will be described separately in a forthcoming publication.

Transport assays. To measure, optimally, the *glpT*-encoded G3P transport system, cells were first grown on DYT medium (21) in the presence of 0.2% glycerol or in minimal medium A (21) containing 0.2% glycerol. After growth overnight, the cells were harvested and washed twice in 10 mM Tris-hydrochloride (pH 7.0) containing 150 mM sodium chloride. They were suspended in 1 ml of the same medium at an optical density (578 nm) of 1.0. A 50-μl amount of [*U*-^{14}C]G3P (120 mCi/mmol, New England Nuclear Corp.) was added to a final concentration of 0.3 μM, and portions of 100 or 200 μl each were filtered through a membrane filter (0.65-μm pore size; Millipore Corp.) at different time intervals. The filters were next washed with 10 ml of the same buffer. All operations were done at room temperature. The filters were dried and counted in a toluene-based scintillation fluid. To measure optimally the *ugp*$^+$-dependent system, cells were first grown overnight on minimal medium A containing 0.2% glucose as carbon source. They were washed and suspended in 10 mM Tris (pH 7.0) containing 25 mM sodium chloride–50 mM potassium phosphate; 2 min before the addition of [^{14}C]G3P (final concentration, 0.3 μM), glucose (final concentration, 0.2%) was added. For the determination of the K_m of the *ugp*$^+$-dependent G3P transport system, various amounts of unlabeled G3P were added 20 s before the addition of [^{14}C]G3P, and three 100-μl portions were filtered within 16 s.

For the determination of the dependence on pH, ionic strength, possible inhibitors, and stimulatory compounds, cells were first resuspended in a particular suitable buffer, as indicated in the figure legends. The compounds to be tested were next added in small volumes of concentrated solutions 20 s before the addition of [^{14}C]G3P (final concentration, 0.3 μM).

RESULTS

Isolation of Mu*cts* insertions in *glpT*. A series of mutants containing phage Mu*cts* inserted in the *glpT* locus were isolated. This temperature-sensitive bacteriophage can be inserted into the *E. coli* chromosome in a seemingly indiscriminate fashion. Insertion of the prophage in any given gene destroys gene function and, since Mu insertions are absolutely polar, insertion also prevents any expression of genes distal to the insertion in multicistronic operons (3, 4, 13). Accordingly, information regarding gene arrangement within an operon can be obtained by examining the effects of a number of independently isolated phage Mu insertions. TS100 was chosen as starting strain. It is *glpR*, i.e., constitutive for G3P transport. Constitutivity of the *glp* regulon is preferable when isolating *glpT* mutants by fosfomycin, since resistance against this antibiotic can also occur by mutations in several other loci (30). Strain TS100 was lysogenized by phage Mu*cts*, and clones were selected that were resistant to fosfomycin. A total of 40 independent mutants (TS101 to TS140) were isolated. These mutants were able to grow aerobically on glycerol but not on G3P. To ensure that a mutation had taken place within the *glpT* operon (20), strains from the series TS101 to 140 (Table 1) were transduced by using a phage P1 lysate prepared on a *glpT*$^+$ *nalA* strain (LA3429). *nalA* transductants were selected and scored for growth on G3P. The 20 mutants chosen could be transduced to G3P$^+$ and were assumed to contain phage Mu*cts* within the *glpT* operon.

In the phage P1 transduction with strains TS101, TS104, and TS137, resistance to phage Mu was also scored. With strains TS101 and 137, G3P$^-$ transductants were resistant, whereas most of the G3P$^+$ transductants were sensitive. This indicated that the *glpT* genotype of these mutants was indeed caused by phage Mu*cts* insertion in *glpT*. Transductants of strain TS104 were all resistant to phage Mu irrespective of their G3P phenotype. This indicated that the *glpT* mutation in this strain was probably not caused by the insertion of Mu*cts* in *glpT* but by a spontaneous mutation in *glpT* in addition to

the Mu*cts* insertion elsewhere in the chromosome.

For further analysis, we choose *nalA* transductants that still were G3P$^-$. Uptake of G3P by one of the mutants, strain LA5001 (the *nalA* derivative of strain TS101), is shown in Fig. 1 in comparison to its parent strain TS100. As can be seen, the mutation in *glpT* resulted in a complete loss of the ability to take up G3P.

The periplasmic proteins in the osmotic shock fluid of parent and mutants were compared by different analytical gel electrophoretic procedures. Figure 2 shows a slab gel stained after electrophoresis in the presence of SDS. Samples were kept at room temperature in SDS or heated at 100°C in SDS plus dithiothreitol for 10 min. Under these conditions, the GLPT protein is known to alter its electrophoretic mobility and can easily be identified (2).

Two types of mutants were obtained (Fig. 2); 19 mutants such as LA5001 and LA5003 lacked GLPT, and only one, LA5004, still contained it.

Two-dimensional gel electrophoresis, which separates the periplasmic proteins much better, is shown in Fig. 3. By this technique, it could be confirmed that all mutants but one (LA5004) were unable to produce GLPT.

Reversion of the *glpT*::Mu*cts* strains to a G3P$^+$ phenotype. About 10^9 cells of each of the

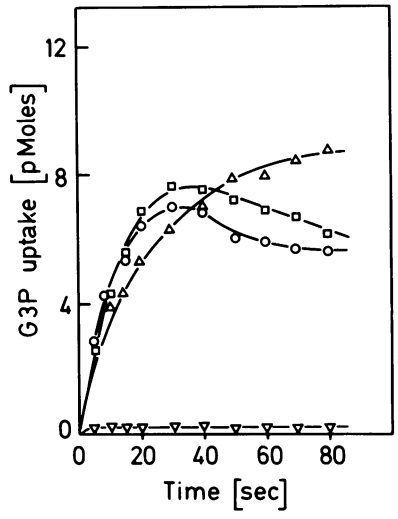


FIG. 1. *G3P uptake in wild-type, mutant, and revertant strains. Results are given as amount of G3P taken up per 50-μl portion of bacterial suspension. Symbols:* (△) *wild-type strain TS100;* (▽) *glpT::Mucts strain TS101;* (○) *revertant strain LA5301; and* (□) *ugp$^+$ transductant LA3427. Wild-type and glpT::Mucts strains were tested under conditions optimal for the glpT-encoded G3P transport system; the revertant and the ugp$^+$ transductant were tested under conditions optimal for the ugp$^+$-encoded system.*

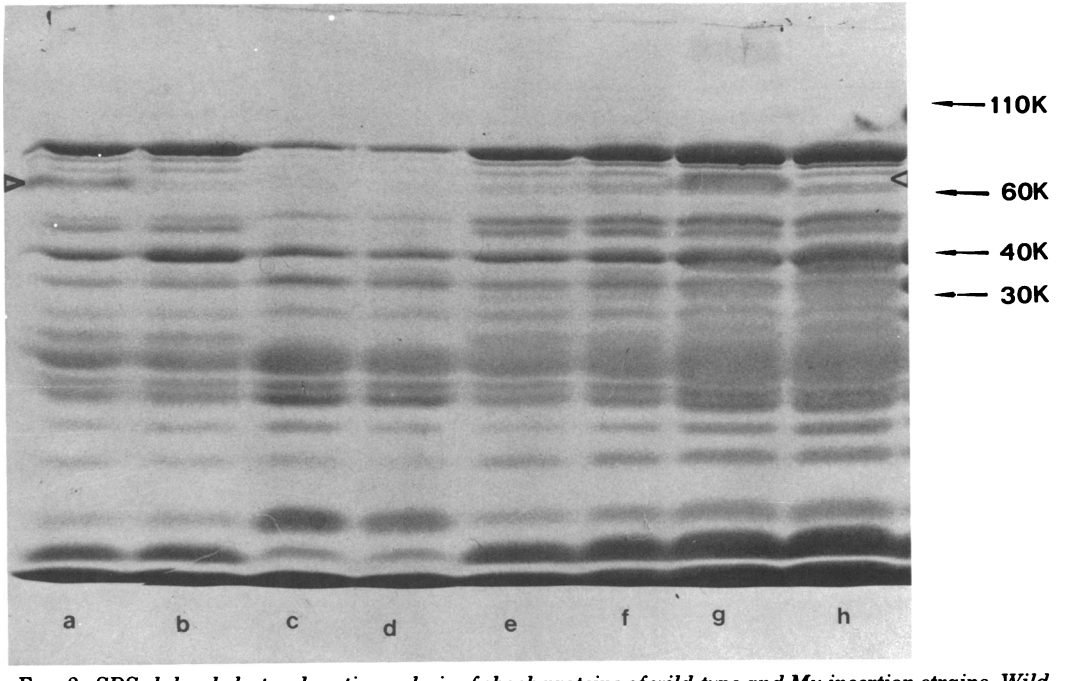
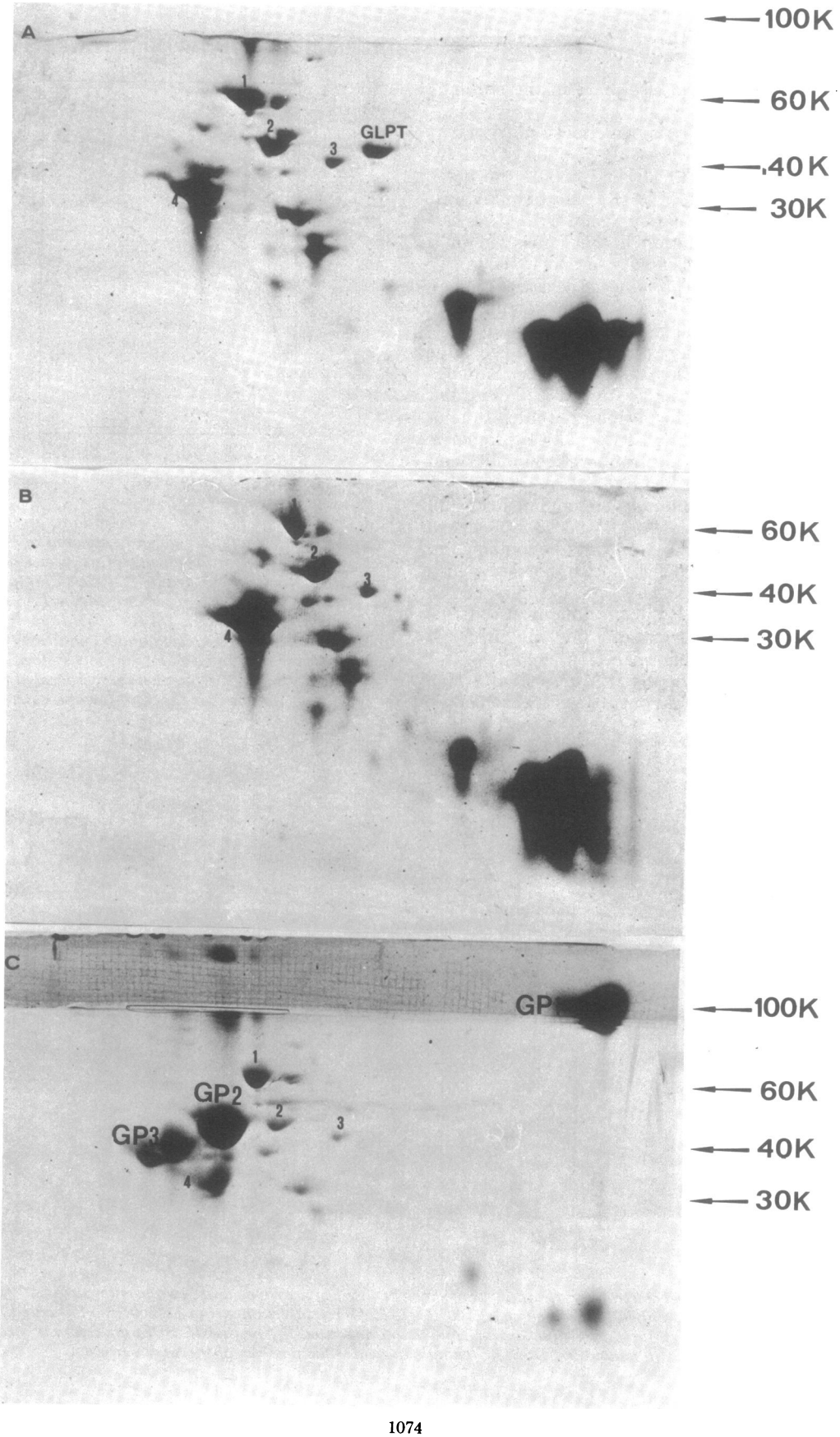


FIG. 2. *SDS slab gel electrophoretic analysis of shock proteins of wild-type and Mu insertion strains. Wild-type strain TS100: (a) unheated; (b) heated at 100°C. glpT::Mucts insertion strain LA5001: (c) unheated, (d) heated in SDS. glpT::Mucts insertion strain LA5037: (e) unheated, (f) heated in SDS. glpT Mucts strain LA5004: (g) unheated, (h) heated in SDS. The triangle indicates the position of the GLPT protein.*


A
1
2
3
4
GLPT
100K
60K
40K
30K
B
2
3
4
60K
40K
30K
C
GP
1
GP2
2
3
GP3
4
100K
60K
40K
30K

glpT::Mu*cts* strains were plated on minimal plates containing G3P as the sole source of carbon and incubated at 43°C for 4 days. After 1 to 2 days, very small, temperature-resistant colonies appeared that were still G3P$^-$. After another 2 to 3 days, 10 to 15 clones appeared that had become G3P$^+$. The reversion was therefore a two-step process. Three to four independent revertants of each mutant were isolated, and some of them were analyzed further. All of the revertants were still lysogenic for phage Mu, i.e., they released Mu. Phage P1 lysates were grown on these revertants and were used to transduce *nalA* into strain LA3430 (it did not grow on G3P and did not produce GLPT). From a total of 26 revertants, the G3P$^+$ phenotypes of only three (LA5204, LA5109, and LA5107) were cotransducible with *nalA*, and were therefore probably located within the *glpT* operon. Electrophoretic analysis of the shock proteins of these *glpT*-linked revertants revealed the presence of a GLPT protein that was indistinguishable from the wild-type GLPT protein (not shown). Since the number and the order of the genes within the *glpT* operon are not yet known, it is not clear whether these revertants arose from excision of Mu either from the structural gene for the GLPT protein or from a hypothetical promoter proximal gene in the *glpT* operon.

The phage P1 transduction data showed that the majority of revertants arose from a secondary mutation outside the *glpT* operon. However, with one exception (LA5104), phage P1 lysates of these revertants were unable to transduce an *nalA*-linked G3P$^-$ phenotype into a wild-type strain. This inability is due either to zygotic induction of phage Mu or the inability of phage P1 to package *nalA* plus *glpT*::Mu.

Transport assays for G3P using these revertants revealed the presence of an efficient transport system. Figure 1 shows the uptake of one of the revertants, LA5301 (a revertant of strain LA5001). As can be seen, under optimal conditions, the initial rate of G3P uptake in the revertant is even higher than that in the parent strain, TS100.

Gel electrophoretic analysis of periplasmic proteins of the revertant strains that do not map within *glpT*. Separation on SDS-polyacrylamide slab gels of the periplasmic proteins of the revertant LA5301 and its parent LA5001 is shown in Fig. 4. Major changes in the protein patterns can be observed. Several proteins have disappeared or are reduced in their amounts. The GLPT protein is still lacking, but three new proteins have appeared. This can also clearly be recognized by separation on two-dimensional polyacrylamide gel electrophoresis (Fig. 3). To indicate their function (ability to restore G3P transport), we designated these proteins GP 1, 2, and 3. Looking at several other revertants that do not map in *glpT*, it became clear that all of them contained these three proteins. (In some revertants, as shown in Fig. 3, a fourth protein appeared. Since this protein is not present in all revertants as well as in other strains that have the new transport system, it is apparently unrelated to the G3P$^+$ phenotype of these strains.) One of the revertants was of particular interest. It was derived from the Mu insertion strain LA5004, the only one that still contained GLPT. From this mutant, two types of revertants were obtained. One (LA5204) was a true revertant in *glpT*. Its periplasmic proteins were identical to the parent strain TS100. The other (LA5104) contained, in addition to GLPT, GP 1, 2, and 3. In contrast to all other revertants that we analyzed, its *glpT* genotype could be transduced into a *glpT*$^+$ recipient resulting in the appearance of an *nalA*-linked G3P$^-$ phenotype. This again demonstrates that strain LA5004 from which LA5104 was derived is the result of two mutational events, a Mu*cts* insertion outside the *glpT* operon and a spontaneous mutation in *glpT*. The latter is transducible with *nalA* by phage P1 transduction.

Genetic analysis of the G3P$^+$ revertants. Since most of the G3P$^+$ revertants did not map within the *glpT* operon, three Hfr crosses were performed to locate the position of the reversion on the genetic map. By two different Hfr crosses with Hfr G6 and KL16 as donors and LA5301 as recipient, a linkage of the G3P$^+$ phenotype to the *araD* marker was indicated. To substantiate such a linkage, a transduction was performed with a phage P1 lysate that was grown on an *ara*$^+$ strain (LA3400). Of 43 *ara*$^+$ transductants, 38 were found to be G3P$^-$, and 5 were G3P$^+$. Thus, the mutation that leads to the appearance of the G3P$^+$ phenotype must be closely linked to the arabinose operon. We call this locus *ugp*$^+$ for "uptake of glycerol phosphate."

To ensure that this mutation is indeed responsible for the appearance of GP 1, 2, and 3 in the

FIG. 3. *Two-dimensional polyacrylamide gel electrophoretic analysis of shock proteins of wild-type, Mu insertion strain, and revertant. Osmotic shock fluid was obtained from wild-type strain TS100 (A), glpT::Mucts strain LA5001 (B), and revertant strain LA5301 (C). The spots designated 1 to 4 are marked for orientation purpose. Separation occurs in the first dimension (left to right) in urea and in the second dimension (top to bottom) in SDS. A 300-μg amount of protein was applied.*

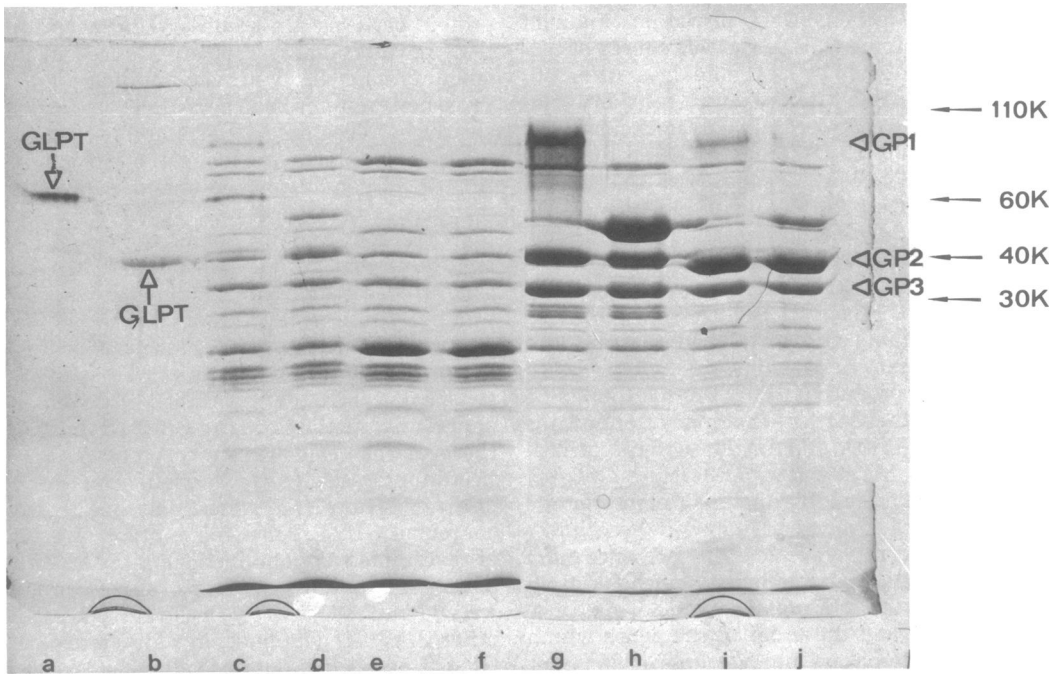


FIG. 4. *SDS slab gel electrophoretic analysis of shock proteins of different strains. (a) Unheated sample of purified GLPT protein; (b) purified GLPT protein after heating at 100°C in the presence of SDS; (c) proteins from strain LA5000 (wild-type, transduced to nalA); (d) SDS-heated preparation of (c); (e) glpT::Mucts insertion strain LA5001; (f) SDS-heated sample of (e); (g) reversion strain LA5301; (h) SDS-heated sample of (g); (i) strain LA108; (j) SDS-heated sample of (i).*

periplasm, the shock proteins of two *ara*$^+$ transductants were analyzed by two-dimensional polyacrylamide gel electrophoresis. It was found that the loss of the G3P$^+$ phenotype in this transduction resulted in the disappearance of GP 1, 2, and 3 (not shown).

F′ episomes were transferred into the revertant strain LA5301. These episomes carried mutations in *ara* other than *araD* but were supposedly wild type in respect to *ugp* (*ugp*°). ARA$^+$ merodiploids were isolated and screened for growth on G3P. Merodiploids obtained with all three donors (Table 1) were G3P$^-$ and did not produce proteins GP 1, 2, and 3. This indicates that the mutation leading to the appearance of the new G3P transport system has occurred within a gene that might code for a cytoplasmic repressor.

Presence of GP 1, 2, and 3 in other *E. coli* strains. From the similar appearance of proteins GP 1, 2, and 3 in all of our strains where the reversion was not linked to *glpT*, it seemed that this reversion results in the derepression of a system that is either dormant or not induced in the wild-type *E. coli* strain. We therefore searched for strains that would contain these proteins under normal growth conditions. A survey of two-dimensional polyacrylamide gels of shock fluids obtained from many unrelated strains revealed the presence of proteins GP 1, 2, and 3 in strain LA108 (Fig. 4), a derivative of BUG-6. This strain is a K-12 derivative. It grows and divides normally at 35°C, but forms long filaments at 42°C (23). The *bug* mutation does not affect DNA replication but does affect septum formation. When grown at the permissive temperature, this strain also contains GLPT. To correlate the presence of GP 1, 2, and 3 to the transport of G3P in this strain, we inserted, by Hfr conjugation with strain LA3431, a defective *glpT* operon into this strain. The donor is *nalA glpT* and does not produce GLPT. Two types of *nalA glpT* recombinants were obtained. One was G3P$^+$, and the other was G3P$^-$. Both recombinants still exhibited the BUG phenotype. Proteins GP 1, 2, and 3 appeared only in the G3P$^+$ recombinants (Fig. 5). Thus, it seems likely that the appearance of GP 1, 2, and 3 in strain BUG-6 is also connected to G3P transport.

Accidentally, we found that derepression of GP 1, 2, and 3 is not only obtained by selecting the Mu*cts*::*glpT* strains for growth on G3P. We had transduced our parent strain, TS100, to *nalA* by phage P1 transduction. Gel electropho-

retic analysis of this *nalA* derivative (strain LA5000) clearly showed, beside the normal amount of GLPT, the additional presence of small amounts of GP 1 in the periplasm (Fig. 4). Traces of GP 2 and 3 could also be detected in this strain by two-dimensional gel electrophoresis (not shown).

Preliminary characterization of GP 1, 2, and 3. When heated in SDS, GP 1 dissociated into a polypeptide chain of an approximate molecular weight of 50,000 (Fig. 4). This dissociation required the presence of dithiothreitol, indicating the participation of interchain s-s bridges in the native protein complex (data not shown). In crude preparations, when analyzed by two-dimensional gel electrophoresis, GP 1 could dissociate into the polypeptide chain during separation in the first dimension even in the absence of dithiothreitol. In contrast, purified GP 1 analyzed by this technique migrated as a single spot at its usual high-molecular-weight position. However, heating in urea at 100°C in the presence of dithiothreitol dissociated the protein entirely in the polypeptide chain that again migrated as a single spot with a molecular weight of about 50,000 (not shown). Even though this behavior was reminiscent of GLPT (Fig. 4), GP 1 did not cross-react with anti-GLPT antibodies.

From strain LA108, we purified proteins GP 1 and GP 2 to apparent homogeneity and protein GP 3 to a state free of proteins GP 1 and GP 2. Binding tests by equilibrium dialysis using [^{14}C]G3P as substrate revealed that GP 2 is a binding protein for G3P with a K_d in the micromolar range (M. Argast, manuscript in preparation). Proteins GP 1 and GP 3 did not exhibit any binding affinity. By gel electrophoretic analysis in SDS (Fig. 4), proteins GP 1, 2, and 3 of the revertant and the unrelated strain LA108 appeared to be identical in their molecular weights. For protein GP 2, this could also be demonstrated by using an analytical method that separates proteins according to electrical charge.

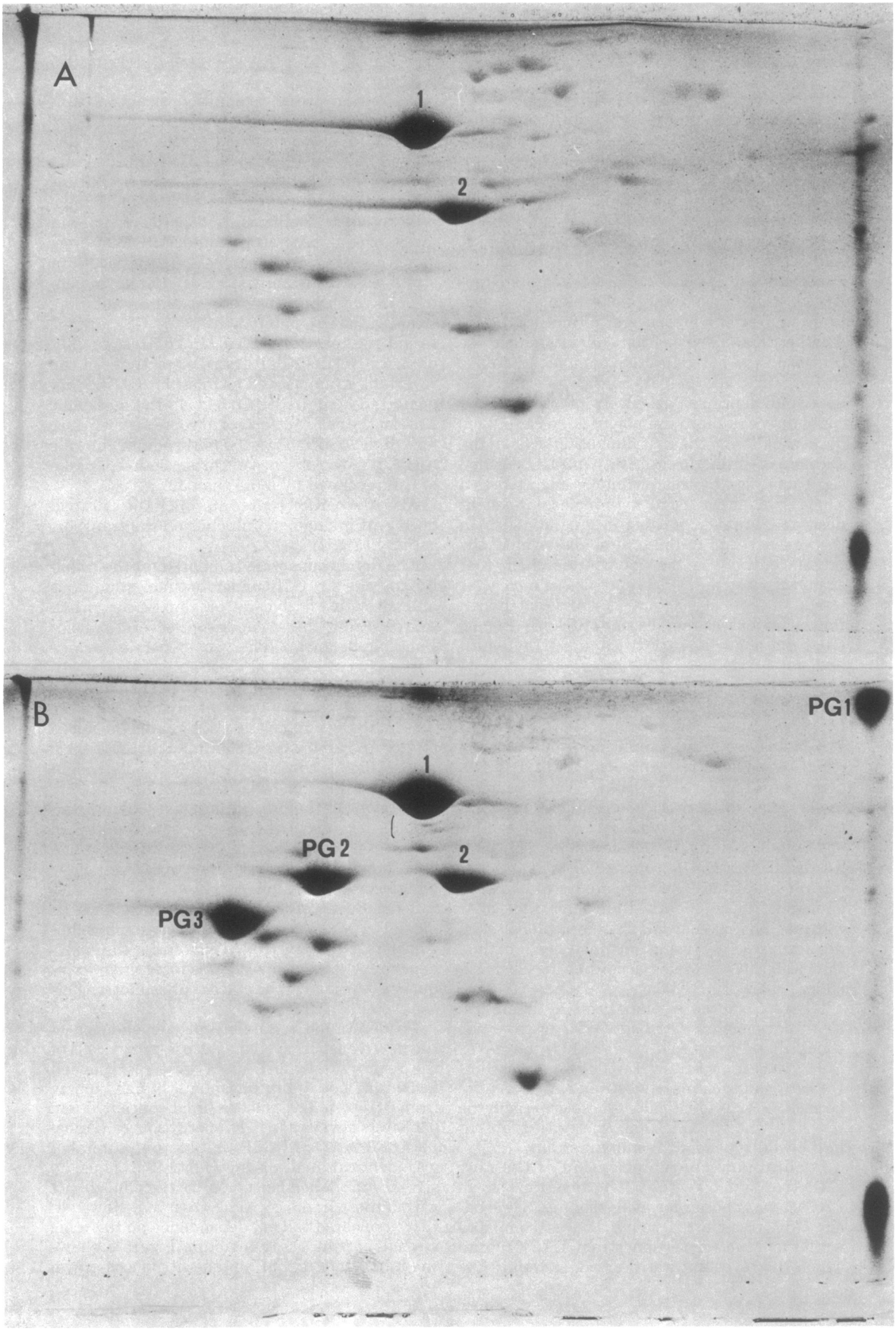
Figure 6 shows a comparison by analytical electrofocusing of purified GP 2 with crude shock fluids from wild-type TS100, the *glpT*::Mu*cts* insertion strain LA5001, its revertant LA5301, and the unrelated strain LA108. As can be seen, GP 2 is identical by these criteria in the revertant and strain LA108, whereas it is absent in the wild-type and the mutant strain.

Preliminary characterization of the new G3P transport system. The revertant strain LA5301 was chosen for analyzing *ugp*$^+$-dependent G3P transport activity. It was compared with the Mu*cts* insertion strain LA5001 from which the revertant was derived, as well as the parent strain TS100. In addition, strain LA3427, a derivative of strain LA108 that was made *glpT* by Hfr cross, was included in this comparison. As will be shown below, the *glpT*-encoded and the *ugp*$^+$-encoded transport system for G3P exhibited different requirements for optimal transport activity. Therefore, transport activity was measured in these strains under their optimal conditions (Fig. 1). As can be seen, the revertant strain was highly active in G3P uptake, in contrast to the Mu*cts* insertion strain. In addition, strain LA3427 exhibited the same G3P uptake as did the revertant strain. In contrast to G3P uptake in TS100, G3P transport activity in the revertant strain as well as in strain LA3427 appeared to be quite dependent on the metabolic state of the cell. When kept at room temperature for 2 h in 10 mM Tris–150 mM sodium chloride (pH 7.0) in the absence of carbon source, uptake of G3P declined by more than 50%.

Also, the carbon source on which the cells had been growing had an influence on the transport activity (Fig. 7).

The four strains were compared for their ability to take up G3P under optimal conditions after growth on glycerol or glucose as carbon source. From this comparison, several conclusions can be drawn. The *ugp*$^+$-dependent transport activity for G3P in these strains was constitutive and more active when the cells had been growing on glucose than on glycerol. Glucose present in the uptake assay stimulated the initial rate of uptake for G3P. G3P uptake in TS100, a strain that is constitutive for *glpT*-dependent transport, was somewhat sensitive to catabolite repression by the growth on glucose. However, glucose present in the assay medium inhibited G3P uptake more severely when the cells had been growing on glucose than when they grew on glycerol. This phenomenon is sometimes called inducer exclusion and is thought to be mediated via the glucose-inducible phosphotransferase system (25). Apparently, the *ugp*$^+$-dependent transport system is insensitive to inducer exclusion via the glucose phosphotransferase system.

To determine a possible pH dependence of the new transport system, glucose-grown cells of the revertant strain LA5301 were resuspended in 50 mM potassium phosphate buffer ranging from pH 5.5 to 8.0; glucose (final concentration, 0.2%) was always added as energy source. Transport of 3×10^{-7} M G3P (initial rate of uptake) was identical in all tests within ±10%. Thus, no significant dependence on extracellular pH within these limits could be observed. However, there appeared to be a dependence on the ionic strength of the assay medium. Figure 8 shows the initial rate of G3P uptake in glucose-grown


A
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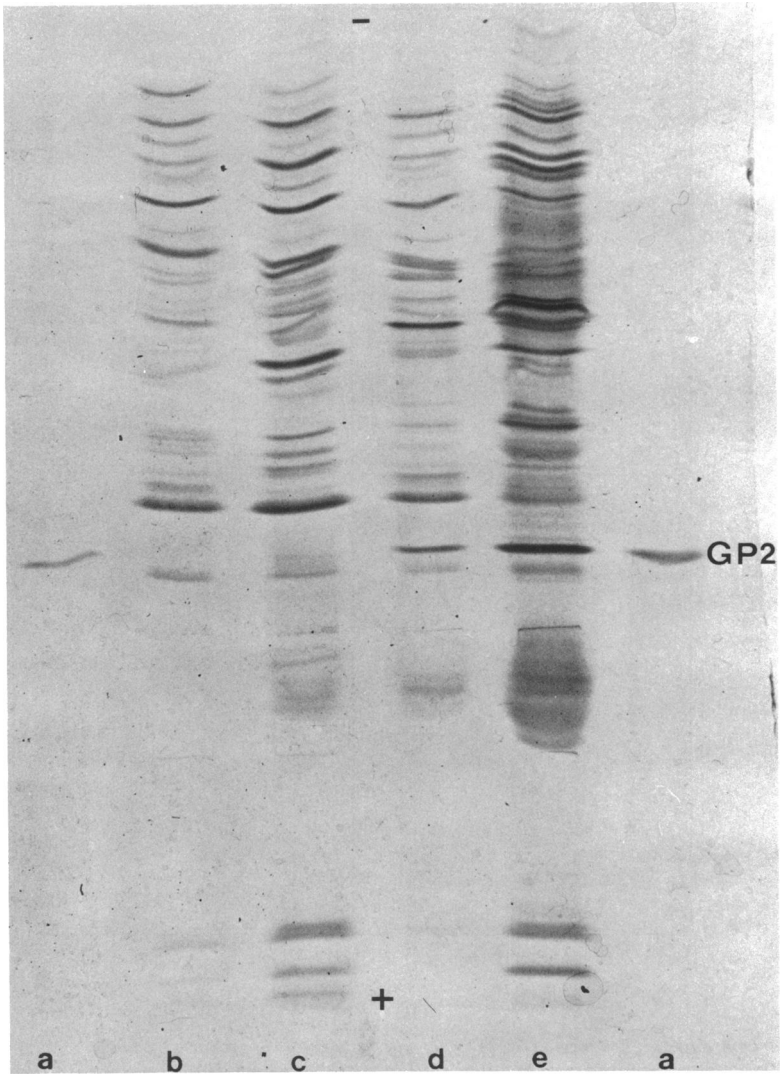

FIG. 6. *Analytical electrofocusing of shock fluids from different strains. (a) Pure GP 2 protein; (b) wild-type TS100; (c) glpT::Mucts strain LA5001; (d) revertant strain LA5301; (e) glpT ugp$^+$ recombinant LA3432. Electrofocusing was performed in commercially available 5% polyacrylamide gels containing ampholines (pH 3.5 to 9) according to the recommendations of the manufacturer (LKB).*

cells suspended in 10 mM Tris-hydrochloride (pH 7.0) and 0.2% glucose containing increasing concentrations of sodium chloride. As can be seen, transport was highest at about 75 mM sodium chloride.

From the observation that all revertants contained the same proteins, GP 1, 2, and 3, one would conclude that the "new transport system" is present also in the wild type, but that it needs to be induced by its proper substrate. Therefore, we examined a possible inhibition of the G3P uptake by other negatively charged organic acids as well as sodium phosphate.

Table 2 lists the compounds that we tested for their ability to inhibit the uptake of 0.3 μM G3P in cells that had been freshly suspended in 10 mM Tris-hydrochloride (pH 7.0) containing 75 mM NaCl. Addition of glucose was omitted in

FIG. 5. *Two-dimensional polyacrylamide gel electrophoretic analysis of shock proteins from two recombinants. The ugp$^+$ glpT$^+$ strain LA108 was crossed with LA3430 (Hfr glpT ugp°). (A) strain LA3433 (glpT ugp°); (B) strain LA3432 (glpT ugp$^+$). Symbols and conditions are as described in the legend to Fig. 3.*

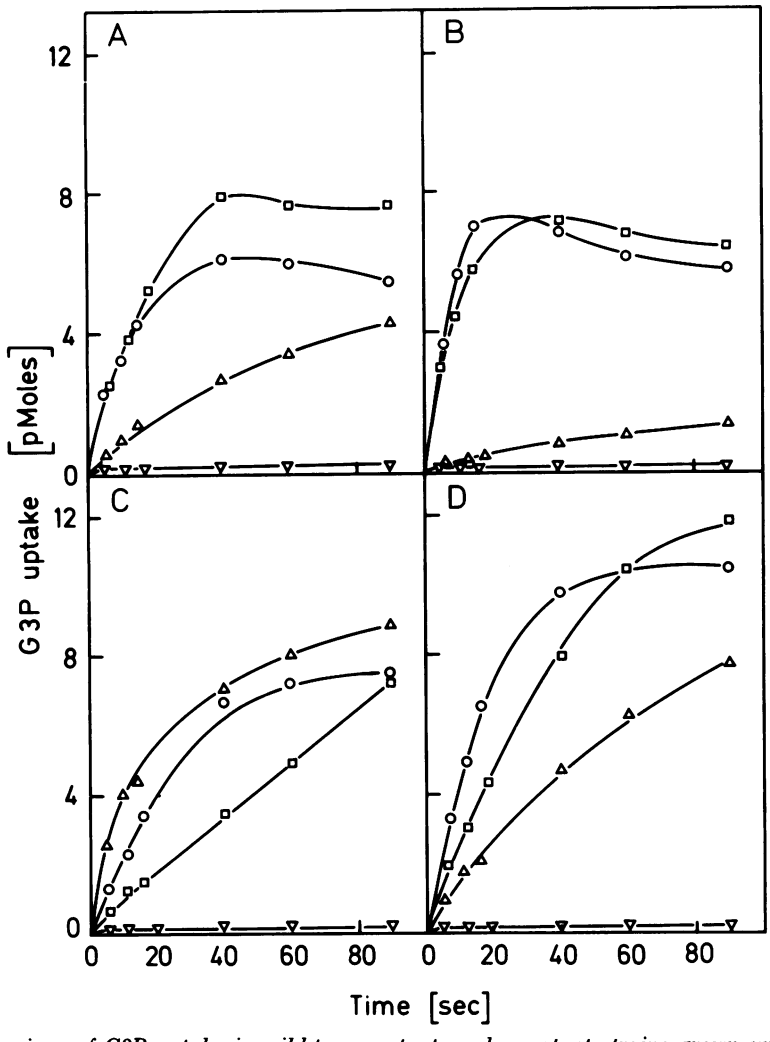

FIG. 7. *Comparison of G3P uptake in wild-type, mutant, and revertant strains grown under different conditions. Cells were grown in minimal medium containing glycerol (A and C) or glucose (B and D) as sole carbon source. They were assayed under conditions optimal for the ugp$^+$-encoded system (A and B) or the glpT-encoded system (C and D). The results are given as amount G3P taken up per 50 μl of cell suspension. Symbols:* (△) *wild-type strains TS100;* (▽) *glpT::Mucts strain LA5001;* (○) *revertant strain LA5301;* (□) *ugp$^+$ recombinant LA3427.*

order to recognize stimulatory effects due to energization. As can be seen, none of these negatively charged compounds exhibited significant inhibition. Even fosfomycin and phosphate, known inhibitors of the *glpT*-dependent system, did not act as inhibitors. The stimulation seen with gluconate was probably due to energization by metabolism.

Transport activity mediated by the *ugp*$^+$ system varied from batch to batch. Cell suspensions of high uptake capacity could hardly be stimulated by the addition of glucose or ions, whereas cells that exhibited lower uptake capacity could be stimulated greatly by the addition of glucose. Sodium ions and, even better, potassium ions also stimulated transport activity. These variations are not yet understood but probably reflect the physiological state of the different batches. The least amount of variation occurred with the 10 mM Tris-25 mM NaCl-50 mM potassium phosphate assay medium (pH 7.0) containing 0.2% glucose.

Using these conditions, the initial rate of uptake was measured at different G3P concentrations. Figure 9 shows the data plotted according to Lineweaver and Burk. As shown, a K_m of 5

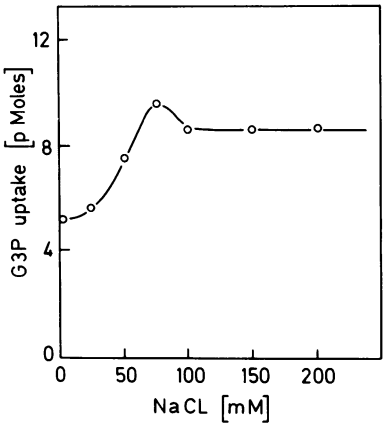

FIG. 8. *Dependence of G3P uptake in a ugp⁺ strain on the ionic strength of the assay medium. Revertant strain LA5301 was grown in minimal medium with glucose as carbon source. Cells were washed and resuspended at an optical density of 1.0 (578 nm) in 10 mM Tris-hydrochloride (pH 7.0) containing increasing amounts of sodium chloride. Two minutes before the addition of 0.3 μM [^{14}C]G3P, 0.2% glucose was added. Other details are as in the legend to Fig. 1.*

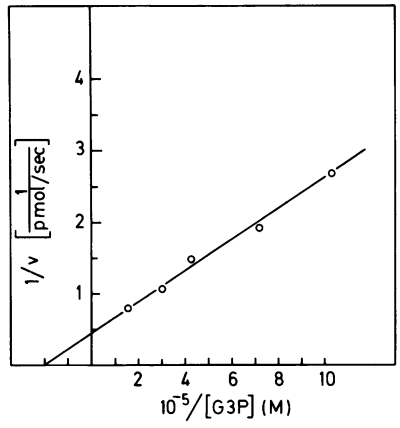

FIG. 9. *Apparent K_m of G3P uptake in a ugp⁺ strain. Revertant strain LA5301 was grown in minimal medium containing glucose as carbon source. G3P uptake was measured under optimal conditions. Initial rate was measured by taking three samples within 16 s. The results are given as amount of G3P taken up per 100 μl of cell suspension (optical density 1.0) per s at room temperature.*

TABLE 2. *Influence of some compounds on G3P uptake in a ugp⁺ revertant*

Compound	Concn	Activity (% of control)
Glyceraldehyde-3-phosphate	1 mM	93
Succinate	1 mM	86
Glucose-6-phosphate	1 mM	88
Gluconate	1 mM	180
Fumarate	1 mM	78
Fosfomycin	8 mM	93
Phosphoenolpyruvate	5 mM	105
Glutamate	1 mM	106
Aspartate	1 mM	112
Phosphate	50 mM	115
Isoleucin	1 mM	99

$\times 10^{-6}$ M can be extrapolated.

DISCUSSION

G3P⁻ mutants were isolated that have phage Mu*cts* inserted in *glpT*, an operon that codes for a transport system for G3P.

All 19 *glpT*::Mu*cts* strains lack GLPT, a protein that had previously been implicated in the transport of G3P (1, 29) and that is coded for by a gene within the *glpT* operon (26). From the fact that, of 19 *glpT*::Mu*cts* insertions, none was found that still contained GLPT, together with the observation that Mu insertions are strongly polar (4), one would conclude that the structural gene for GLPT is the last in a polycistronic operon. G3P⁺ revertants were obtained from these mutants. The majority of these revertants revealed the existence of a hitherto unknown transport system for G3P. This was clear from the following circumstances: (i) phage Mu was still positioned within *glpT*; (ii) the mutation *ugp*⁺ leading to the G3P⁺ phenotype was cotransducible with *araD* by phage P1 transduction, i.e., far removed from the *glpT* region; and (iii) the specificity and regulation of the new transport system was entirely different from that encoded by *glpT*. Diploid strains that carry *ugp*° and *ugp*⁺ have a G3P⁻ phenotype. This indicates that the *ugp*⁺ mutation has affected a cytoplasmic repressor.

A preliminary characterization of the new transport system reveals the following. It has a K_m for G3P of 5 μM, in comparison to 12 μM for the *glpT*-encoded system. Fosfomycin and phosphate, known inhibitors of the *glpT*-encoded system, have no substrate properties. In contrast to the *glpT*-encoded system, it is not subjected to catabolite repression and "inducer exclusion" (25) by glucose. Most interesting, however, and simultaneous with the appearance of the new transport system, three hitherto unknown proteins (GP 1, 2, and 3) can be found in the osmotic shock fluid of these strains, of which one is a highly specific binding protein for G3P.

It is surprising that we had never observed the appearance of this new transport system, including the three periplasmic proteins, when

reverting spontaneous mutants in *glpT* (29). Possibly the very presence of Mu*cts* in *glpT* or somewhere else in the chromosome is responsible for the occurrence of the reversion. After heat induction, Mu might insert itself at a secondary location that gives rise to *ugp*$^+$ and at the same time remains in its original place (19). It seems that Mu*cts* does not have to be inserted in *glpT* to give rise to *ugp*$^+$ upon selection on G3P. Strain LA5004 is most likely a point mutant in *glpT* and carries Mu*cts* somewhere else in the chromosome. However, reversion at 43°C on G3P plates gives rise to *ugp*$^+$. A detailed genetic analysis of the *ugp*$^+$ mutation will be necessary to clarify whether or not Mu is involved in this reversion.

Other transport systems have been found to effectively transport a seemingly unrelated substrate only after a mutation that changed its regulation to constitutivity (11, 17, 20, 24). From the observation that all *ugp*$^+$ revertants produce the same three periplasmic proteins, it appears likely that *ugp*$^+$ is a regulatory mutation of an already existing transport system of unknown specificity. This system is either not induced under our growth conditions or is cryptic, as in the case of the 2-keto-3-desoxy-D-gluconate transport system (17).

The *ugp*$^+$-dependent G3P transport system did not appear to be cryptic or uninduced in all strains. Strain LA108, which is entirely unrelated to the TS100 derivatives, did contain GP 1, 2, or 3 in its periplasm when grown under usual conditions. Moreover, after the introduction of *glpT* into this strain, it exhibited transport activity for G3P that was very similar to that of the *ugp*$^+$ reversion strains. Also, we found that the introduction of the *nalA* marker into the wild-type strain TS100 by phage P1 transduction gave rise to the synthesis of small amounts of GP 1, 2, and 3. The significance of this phenomenon is, at present, not clear.

Several gene loci related to transport systems are located in close vicinity to *ugp*$^+$: *araC*^{c}, affecting transport of arabinose and lactose (20); *xtl*^{s}, responsible for constitutive fructose transport (24); *brnS*, affecting transport of branched-chain amino acids (10); and *trkC*, necessary for the retention of potassium (7). It seems unlikely that these mutations are related to *ugp*$^+$, since none of their substrates inhibited uptake of G3P via the *ugp*$^+$-dependent system. Moreover, none of the negatively charged compounds listed in Table 2 showed any inhibitory activity.

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LITERATURE CITED

1. **Argast, M., G. Schumacher, and W. Boos.** 1977. Characterization of a periplasmic protein related to *sn*-glycerol-3-phosphate transport in *Escherichia coli*. J. Supramol. Struct. **6**:135–153.
2. **Boos, W., I. Hartig-Beecken, and K. Altendorf.** 1977. Purification and properties of a periplasmic protein related to *sn*-glycerol-3-phosphate transport in *Escherichia coli*. Eur. J. Biochem. **72**:571–581.
3. **Bukhari, A. J.** 1976. Bacteriophage Mu as a transposition element. Annu. Rev. Genet. **10**:389–411.
4. **Bukhari, A. J., and D. Zipser.** 1972. Random insertion of Mu-1 DNA within a single gene. Nature (London) New Biology **236**:240–243.
5. **Cardelli, J., and J. Konisky.** 1974. Isolation and characterization of an *Escherichia coli* mutant tolerant to colicins Ia and Ib. J. Bacteriol. **119**:379–385.
6. **Cozzarelli, N. R., W. B. Freedberg, and E. C. C. Lin.** 1968. Genetic control of the L-α-glycerophosphate system in *E. coli*. J. Mol. Biol. **31**:371–387.
7. **Epstein, W., and B. S. Kim.** 1971. Potassium transport loci in *Escherichia coli* K-12. J. Bacteriol. **108**:639–644.
8. **Freedberg, W. B., and E. C. C. Lin.** 1973. Three kinds of controls affecting the expression of the *glp* regulon in *Escherichia coli*. J. Bacteriol. **115**:816–823.
9. **Garen, A., and C. Levinthal.** 1960. A fine structure genetic and chemical study of the enzyme alkaline phosphatase of *E. coli*. Biochim. Biophys. Acta **38**: 470–483.
10. **Guardiola, J., M. DeFelice, T. Klopotowski, and M. Jaccarino.** 1974. Mutations affecting the different transport systems for isoleucine, leucine, and valine in *Escherichia coli* K-12. J. Bacteriol. **117**:393–405.
11. **Hacking, A. J., and E. C. C. Lin.** 1977. Regulatory changes in the fucose system associated with the evolution of catabolic pathway for propanediol in *Escherichia coli*. J. Bacteriol. **130**:832–838.
12. **Hayashi, S., J. P. Koch, and E. C. C. Lin.** 1964. Active transport of L-α-glycerophosphate in *Escherichia coli*. J. Biol. Chem. **239**:3098–3105.
13. **Howe, M. M., and E. G. Bade.** 1975. Molecular biology of bacteriophage Mu. Science **190**:624–632.
14. **Johnson, W. C., T. J. Silhavy, and W. Boos.** 1975. Two-dimensional polyacrylamide gel electrophoresis of envelope proteins of *Escherichia coli*. Appl. Microbiol. **29**:405–413.
15. **Kistler, W. S., and E. C. C. Lin.** 1971. Anaerobic L-α-glycerophosphate dehydrogenase of *Escherichia coli*: its genetic locus and its physiological role. J. Bacteriol. **108**:1224–1234.
16. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
17. **Lagarde, A. E., J. M. Pouysségur, and F. R. Stoeber.** 1973. A transport system for 2-keto-3-desoxy-D-gluconate uptake in *Escherichia coli* K-12. Eur. J. Biochem. **36**:328–341.
18. **Lin, E. C. C.** 1976. Glycerol dissimilation and its regulation in bacteria. Annu. Rev. Microbiol. **30**:535–578.
19. **Ljungquist, E., and A. I. Bukhari.** 1977. State of prophage Mu DNA upon induction. Proc. Natl. Acad. Sci. U.S.A. **74**:3143–3147.
20. **Messer, A.** 1974. Lactose permeation via the arabinose transport system in *Escherichia coli* K-12. J. Bacteriol. **120**:266–272.
21. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
22. **Neu, H. C., and L. A. Heppel.** 1965. The release of

enzymes from *Escherichia coli* by osmotic shock and during the formation of spheroplasts. J. Biol. Chem. **240**:3685-3692.

23. **Reeve, J. N., D. J. Groves, and D. J. Clark.** 1970. Regulation of cell division in *Escherichia coli*: characterization of temperature-sensitive division mutants. J. Bacteriol. **104**:1052-1064.
24. **Reiner, A. M.** 1977. Xylitol and D-arabitol toxicities due to derepressed fructose, galactitol, and sorbitol phosphotransferases of *Escherichia coli*. J. Bacteriol. **132**: 166-173.
25. **Saier, M. H.** 1977. Bacterial phosphoenolpyruvate:sugar phosphotransferase systems: structural, functional, and evolutionary interrelationships. Bacteriol. Rev. **41**: 856-871.
26. **Schumacher, G., and K. Bussmann.** 1978. Cell-free synthesis of proteins related to *sn*-glycerol-3-phosphate transport in *Escherichia coli*. J. Bacteriol. **135**:239-250.
27. **Shen, B. H. P., and W. Boos.** 1973. Regulation of the β-methyl-galactoside transport system and the galactose-binding protein by the cell cycle of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **70**:1481-1485.
28. **Silhavy, T. J., M. J. Casadaban, H. A. Shuman, and J. R. Beckwith.** 1976. Conversion of β-galactosidase to a membrane-bound state by gene fusion. Proc. Natl. Acad. Sci. U.S.A. **73**:3423-3427.
29. **Silhavy, T. J., I. Hartig-Beecken, and W. Boos.** 1976. Periplasmic protein related to the *sn*-glycerol-3-phosphate transport system of *Escherichia coli*. J. Bacteriol. **126**:951-958.
30. **Venkateswaran, P. S., and H. C. Wu.** 1972. Isolation and characterization of a phosphomycin-resistant mutant of *Escherichia coli* K-12. J. Bacteriol. **110**:935-944.