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Characteristics of a Binding Protein-Dependent Transport System for *sn*-Glycerol-3-Phosphate in *Escherichia coli* That Is Part of the *pho* Regulon

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The *ugp*-dependent transport system for *sn*-glycerol-3-phosphate has been characterized. The system is induced under conditions of phosphate starvation and in mutants that are constitutive for the *pho* regulon. The system does not operate in membrane vesicles and is highly sensitive toward osmotic shock. The participation of a periplasmic binding protein in the transport process can be deduced from the isolation of transport mutants that lack the binding protein. As with other binding protein-dependent transport systems, this protein appears to be necessary but not sufficient for transport activity. The isolation of mutants has become possible by selection for resistance against the toxic analog 3,4-dihydroxybutyl-1-phosphonate that is transported by the system. *sn*-Glycerol-3-phosphate transported via *ugp* cannot be used as the sole carbon source. Strains have been constructed that lack alkaline phosphatase and glycerol kinase. In addition, they are constitutive for the *glp* regulon and contain high levels of glycerol-3-phosphate dehydrogenase. Despite the fact that these strains exhibit high *ugp*-dependent transport activity for *sn*-glycerol-3-phosphate they are unable to grow on it as a sole source of carbon. However, when cells are grown on an alternate carbon source, ^{14}C label from [^{14}C]*sn*-glycerol-3-phosphate appears in phospholipids as well as in trichloroacetic acid-precipitable material. The incorporation of ^{14}C label is strongly reduced when *sn*-glycerol-3-phosphate is the only carbon source. In the presence of an alternate carbon source, this inhibition is relieved, and *sn*-glycerol-3-phosphate transported by *ugp* can be used as the sole source of phosphate.

Escherichia coli contains two transport systems for *sn*-glycerol-3-phosphate (G3P). One (*glpT* dependent) is part of the *glp* regulon (8). It is induced by glycerol or G3P in the growth medium. Its function is geared toward an efficient degradation of G3P as a carbon source. Accordingly, strains with high *glpT* transport activity also exhibit high activity of aerobic (*glpD*) or anaerobic (*glpA*) dehydrogenase. In addition, the system is highly sensitive to catabolite repression (13). Part of this transport system appears to be a periplasmic protein (GLPT protein) (33), the function of which in transport remains elusive. *glpT*-dependent transport activity can be observed in membrane vesicles, and energy coupling is likely to be mediated via the proton motive force (6). The second transport system was discovered by the isolation of G3P$^+$ revertants of strains that could not grow on G3P due to a defective *glpT*-dependent transport system. These strains were not true revertants, but exhibited a new transport activity for G3P (4) (G3P suppressor strains). The mutation giving rise to this new activity was located close to *araD* on the genetic map of *E. coli* and called *ugp*$^+$. (Ugp$^+$ designates the derepressed state and Ugp^0 designates the repressed state of the *ugp* transport genes.) Even though it is now clear that the precise mapping was incorrect, it is undoubtedly true that the G3P$^+$ suppressor mutations are located outside the original *glpT* locus. (Close linkage of *ugp*$^+$ to *araD* was established by P1 transduction of *araD*$^+$ *ugp*^{0} into the G3P suppressor mutants [*araD ugp*$^+$]. High cotransduction frequency of *araD*$^+$ *ugp*^{0} was observed [4]. It is now clear that the conclusion of cotransduction of these two markers is incorrect, due to a high frequency of *phoB* mutations, defective in a positive regulator of the *pho* regulon. These mutants are phenotypically identical to *ugp*^{0} and are highly selected under anaerobic conditions in a background of constitutivity of the *pho* regulon [39].) Simultaneously with the novel G3P transport activity several new proteins (GP1, 2, 3, 4) appeared in the periplasm of these G3P suppressor strains; one of these proteins was a binding protein with high affinity for G3P. Later on, we found (3) that

the *ugp*-dependent transport activity was also coupled to the appearance of a new outer membrane protein called Ic (18), E (11), or e (37) that recently had been found to be under the control of the *pho* regulon (3, 26, 34). Thus, mutations in *phoR* (14, 36), *phoS* (1, 16) or *phoT* (38) that lead to a constitutive synthesis of alkaline phosphatase also derepress the *ugp*-dependent G3P transport activity (3), with *phoR* and *phoS,T* being most likely identical to the previously reported (28) *nmpA* and *nmpB* mutations, respectively, responsible for the appearance of the new outer membrane protein Ic (E, e). Even though it has been claimed that *nmpA* and *nmpB* are the structural genes for the outer membrane protein (27), this appears to be unlikely from recent results (35).

As presented elsewhere (31a), we were able to map two structural genes (*ugpA* and *ugpB*) of the transport system at 75.3 min of the *E. coli* chromosome.

The isolation of our G3P$^+$ suppressor strains had been done on plates containing 1 mM P_i and 0.2% G3P as the carbon source. It is obvious that such a selection also favors the isolation of mutants constitutive in alkaline phosphatase. This enzyme would split G3P to P_i and glycerol that in turn could be used as the carbon source. Indeed, all of our G3P suppressor strains that are not true *glpT*$^+$ revertants are mutants exhibiting high levels of alkaline phosphate and are in fact *phoR*, *phoS*, or *phoT* mutants. Surprisingly, despite the fact that these strains contained high transport activity for G3P, they were able to grow well on G3P only at P_i concentrations lower than 1 mM. Since alkaline phosphatase is strongly inhibited by P_i concentrations of 100 mM (routinely used in minimal plates), it seemed likely that the presence of this enzyme, and not the G3P transport activity, was responsible for growth on G3P. To elucidate the role and function of the novel G3P transport activity, we constructed several mutants that lack alkaline phosphatase (*phoA*), glycerol kinase (*glpK*), and the *ugp*-dependent transport system and studied their growth properties with G3P as the sole source of carbon and phosphate.

MATERIALS AND METHODS

Bacterial strains, strain construction, growth conditions, and genetic manipulations. All strains and bacteriophages used in the present study are listed in Table 1.

For the study of the physiological role of the *ugp*-dependent G3P transport system, specific strains had to be constructed. The absence of alkaline phosphatese (*phoA*) would prevent splitting of G3P to P_i and glycerol in the periplasm; the absence of glycerol kinase (*glpK*) would prevent utilization of glycerol, but not G3P, as a carbon source; and finally, the absence of the *glpT*-dependent G3P transport system would ensure the *ugp*-dependent system as the only way of G3P entry. For these purposes, strain 4 (*glpK phoA8*) was first made Δ*glpT* by P1 transduction with DL42 (Δ*glpT gyrA*) as the donor and selecting for nalidixic acid resistance. *pho* constitutivity was then introduced by cotransduction of the *phoS,T* mutation of SH114 with a Tn*10* insertion in the nearby *ilv* genes selecting tetracycline resistance (Tc^r) and screening for *ugp*-dependent transport activity, giving strain SH115.

To ensure high levels of G3P catabolic enzymes necessary for the utilization of G3P as a carbon source, the mutation *glpR* was introduced in some of the strains (SH118, SH119). This was achieved via cotransduction of the *glpR* mutation of DL100 with the nearby insertion *zhe-720*::Tn*10*, selecting for Tc^r, and screening *glpD* activity as described below.

Generally, unless otherwise stated, the procedures outlined above were used to construct Δ*glpT*- and *pho*-constitutive strains.

The strains were grown under aeration in minimal medium A (24) or in Tris medium lacking P_i (15) to which P_i was added in the desired amounts. Growth was monitored at 578 nm in an Eppendorf photometer 1101 (cuvettes with 1-cm path length). It was found that an optical density of 1.0 corresponded to approximately 8.5×10^8 cells per ml.

All genetic manipulations, such as P1 transduction and selection for nalidixic acid resistance (*gyrA*), were done as described by Miller (24). Selections of tetracycline-resistant mutants obtained by P1 transduction of Tn*10* was done by plating the cells without phenotypic expression on NB plates (24) containing 3 μg of tetracycline-hydrochloride (Sigma Chemical Co., St. Louis, Mo.) per ml and 20 mM sodium citrate. To score for tetracycline resistance, NB plates containing 20 μg of tetracycline-hydrochloride per ml or minimal plates (24) containing 10 μg of tetracycline-hydrochloride per ml were used. Spontaneous tetracycline-sensitive mutants were isolated by a recently described method (5).

Bacteriophage TC45 sensitivity and adsorption were tested by cross-streaking on R plates (24).

Transport assays. For measurement of *ugp*$^+$-dependent G3P transport, cells were grown logarithmically to an optical density (578 nm) of 0.5 in minimal medium A (24) containing 0.2% glucose as a carbon source. The cells were washed twice and then suspended in the same medium to the same optical density. [*U*-^{14}C]G3P (153 mCi/mmol; New England Nuclear Corp., Boston, Mass.) at a final concentration 0.13 μM was added together with glyceraldehyde-3-phosphate (1 mM final concentration) and glycerol (0.5 mM final concentration). The last two components were added to prevent G3P entry via the *glpT*-encoded G3P transport system and to chase any [*U*-^{14}C]glycerol which might be liberated by the activity of alkaline phosphatase or other phosphatases. Samples (200 μl) were filtered through a membrane filter (0.45-μm pore size, Millipore Corp., Bedford, Mass.) at different time intervals and washed with 10 ml of minimal medium A. All operations were done at room temperature. The filters were dried and counted in a toluene-based scintillation fluid (Lipoluma, LKB).

For the determination of the K_m and V_{max} of the *ugp*$^+$-dependent and the *glpT*-encoded G3P transport system and for the determination of transport inhibi-

TABLE 1. Bacterial strains and bacteriophages

Strain	Parent	Method of isolation	Known markers	Known relevant phenotype	Source/ reference
Bacteria					
LA3430	CSH74		Hfr *glpT*		(4)
SH100	LA3430	Spontaneous Mot$^+$ derivative of LA3430	Hfr *glpT*		This study
SH101	LA3430	Growth on β-glycerol-phosphate	Hfr *glpT ugp$^+$ phoR*	GLPT$^-$, GP (1,2,3)$^+$	This study
SH108	Strain 4	Transduction of strain 4 with P1 grown on DL42	HfrC *glpK ΔglpT gyrA phoA8 ugp^{0a}*	GLPT$^-$	This study
SH114	JF749	Transduction of JF749 with P1 grown on BW711	*xyl lac aroA metB his purE proC cyc rpsL tsx phoT ilv*::Tn*10 ugp$^+$*		This study
SH115	SH108	Transduction of SH108 with P1 grown on SH114	HfrC *glpK ΔglpT gyrA phoA8 phoT ilv*::Tn*10 ugp$^+$*	GLPT$^-$, GP (2,3)$^+$, TC45^s	This study
SH116	SH115	Spontaneous Tc^s	HfrC *glpK ΔglpT gyrA phoA8 phoT ilv ugp$^+$*		This study
SH117	SH116	Transduction of SH116 with P1 grown on SH1200	HfrC *glpK ΔglpT gyrA phoA8 phoT ilv ugpA-704*::Tn*10*	GLPT$^-$, GP (2,3)$^+$, TC45^s	This study
SH118	SH116	Transduction of SH116 with P1 grown on DL100	HfrC *glpR glpK ΔglpT gyrA phoA8 phoT ilv ugp$^+$ zhe-720*::Tn*10*		This study
SH119	SH118	Transduction of SH118 with P1 grown on TS100, selection on glycerol	HfrC *glpR ΔglpT gyrA phoA8 phoT ilv ugp$^+$*		This study
SH1200	SH101	Transduction of SH101 with P1 grown on TS100 with random Tn*10* insertions, obtained by transduction of TS100 with λ NK370::Tn*10*	Hfr *glpT ugpA-704*::Tn*10 phoR*	TC45^s	This study
4			HfrC *phoA8 glpK*		E. C. C. Lin
72			HfrC Δ(*glpR-malA*) *phoA8*	GLPT$^+$	E. C. C. Lin
DL42			*glpR ΔglpT gyrA thi ΔlacU169 araD139*		D. Ludtke
DL100	HfrG6		Hfr *his glpR zhe-720*::Tn*10*		D. Ludtke
BW711			*ΔlacU169 thi strA aroB proC*::Tn*5 ilv*::Tn*10*		B. Wanner
BB26-36			HfrC *glpD3 glpR2 phoA8 tonA22 rel-1* (λ) *plsB26* mutation allowing supplementation with glycerol		(9)
JF749			*xyl lac aroA metB his purE proC cyc rpsL tsx phoS, T ugp$^+$*	TC45^s	J. Foulds
TS100			F$^-$ *araD139 ΔlacU169 thi relA rpsL glpR*	GLPT$^+$	(3)
LA5301			F$^-$ *araD139 ΔlacU169 thi relA rpsL glpR glpT*::*Mucts ugp$^+$*	GLPT$^-$ GP (1,2,3,4)$^+$	(3)
Phages					
TC45				Specific for outer membrane protein Ic (e E)	(7)
λNK370			*b221 cI857 cI171*::Tn*10 o*uga261		N. Kleckner

[a] The superscript^0 indicates the repressed state of the system in contrast to the constitutive state, designated $^+$.

tion by analogs, cells were grown logarithmically to an optical density of 0.5 (578 nm) in minimal medium A (24) containing 0.2% of the appropriate carbon source and then treated as described elsewhere (4). Glucose (20 mM) was present in the assay medium of the *ugp*$^+$ strain.

Incorporation of [*U*-^{14}C]G3P in whole cells. To determine the incorporation of ^{14}C label from G3P in phospholipids and protein, cells were grown in minimal medium A containing 0.4% succinate as a carbon source to an optical density of 0.5 (578 nm). After the addition of 3 μM (final concentration) [*U*-^{14}C]G3P, the cells were incubated for different time intervals at 37°C, and 5-ml samples were precipitated with 10% (final concentration) ice-cold trichloroacetic acid (TCA) and kept on ice for 1 h. The precipitate was washed once with 5% cold TCA. To extract phospholipids 3 ml of chloroform-methanol (2:1, vol/vol) was added, and the suspension was stirred at room temperature for 1 h. After the addition of 1 ml of 50 mM $MgCl_2$ the solute was thoroughly mixed, and the phases were separated by a brief low-speed centrifugation. The chloroform phase was carefully removed and concentrated under nitrogen before subjection to chromatography. After complete removal of the water phase, the pellet was hydrolyzed for 24 to 30 h at 110°C in small volumes (100 to 200 μl) of 6 N HCl. The resulting components were identified by descending paper chromatography (Whatman 3 MM) with *n*-butanol–pyridine–water–acetic acid (15:12:10:3, vol/vol/vol/vol) as the solvent. After drying of the chromatogram, the spots were visualized by autoradiography, which was carried out for 5 days at room temperature with Kodak medical X-ray film.

Phospholipids were identified by thin-layer chromatography on aluminium sheets coated with silica gel (Merck & Co., Inc., Rahway, N.J.), using chloroform-methanol-acetic acid (65:25:8, vol/vol/vol) (22) as the solvent. Radioactive profiles on these thin-layer plates were determined by counting 0.5-cm strips in 5 ml of toluene-based scintillation fluid. Standards were prepared by the incubation of strain BB20-14 (10) for 5 h in minimal A containing 0.2% glucose, 0.004% glycerol, and 5 μCi of [2-^{3}H]glycerol (New England Nuclear Corp.). Phospholipids were extracted and detected as described above.

For determination of the competition by glycerol for the incorporation of G3P, cells of *glpT*$^+$ strain 72 and *ugp*$^+$ strain SH119 were grown in minimal medium A containing 0.4% succinate as the carbon source to an optical density (578 nm) of 0.5. They were labeled at 37°C with 50 μM [^{14}C]G3P. To one-half of the cultures 0.2% glycerol was added before the addition of [^{14}C]G3P. At various time intervals 2-ml samples were removed, precipitated with 10% (final concentration) ice-cold TCA, and kept on ice for 30 min. Total incorporation was measured by filtering a 200-μl sample through Whatman GF/B filters. After washing with 10 ml of 2% TCA, the filters were dried and counted in 5 ml of a toluene-based scintillation fluid (Lipo Luma, LKB). From the remaining precipitate, phospholipids were isolated as described above.

Determination of enzymatic activities. Alkaline phosphatase activity in whole cells was determined by following the enzymatic hydrolysis of p-nitrophenyl phosphate at room temperature, measuring the increase of absorbance at 400 nm. Assay conditions were 1 ml of cells at an optical density (578 nm) of 0.5 in 1 M Tris-hydrochloride (pH 8.0) and 20 μl of 50 mM p-nitrophenyl phosphate (Sigma). The measurement of the activity of aerobic G3P dehydrogenase was performed as described previously (20). Protein was determined by the method of Lowry et al. (23).

RESULTS

G3P entering through the *ugp*-dependent transport system cannot serve as the sole source of carbon, but can serve as the sole source of phosphate. Figure 1 shows growth curves with strain SH115, which carries mutations in *phoA*, *glpK*, and *glpT*, but contains high constitutive levels of *ugp*-dependent G3P transport activity due to a mutation in *phoT*. This strain grows normally on glucose in media containing 100 mM P_i. In contrast, when strain SH115 is suspended in a medium containing 100 mM P_i and G3P as the carbon source no growth can be observed. The addition of glucose to such a stalled culture lets growth resume immediately. It is clear that G3P does not exhibit any toxic effect, since the addition of G3P to cells growing on glucose or on succinate does not reduce their growth rate. The inability of this strain to grow on G3P as the carbon source is also maintained when the P_i concentration is lowered to 0.1 mM (data not shown).

Figure 1 also shows the growth curve of a mutant (SH118) that contains in addition a mutation in *glpR* leading to high constitutive levels of the aerobic G3P dehydrogenase. To exclude any catabolite effect on the possible utilization of G3P the strain was pregrown in succinate. Similar results were obtained. Despite high levels of G3P uptake the strain cannot grow on G3P as the sole source of carbon.

However, as shown in Fig. 1B the same strains can use G3P effectively as the sole source of phosphate. With glucose as the carbon source and 60 μM P_i the strain grows to a final optical density of 0.4. With the additional presence of G3P the strain grows at a normal rate to its usual final optical density of 2.0. As will be seen later, under these conditions the source of phosphate is primarily supplied by G3P after being transported through the *ugp*-dependent transport system.

The ability to grow on G3P is regained in a strain which contains in addition to the *ugp*-dependent system a functioning *glpT*-dependent G3P transport system. Thus, the derepression of the *ugp* system does not prevent utilization of G3P via the *glpT* system.

Inability of *ugp*$^+$ strains to grow on G3P as the only carbon source is not due to a low transport capacity. The inability of *ugp*$^+$ strains to grow on G3P as the sole source of carbon may simply be due to an insufficient maximal rate of G3P

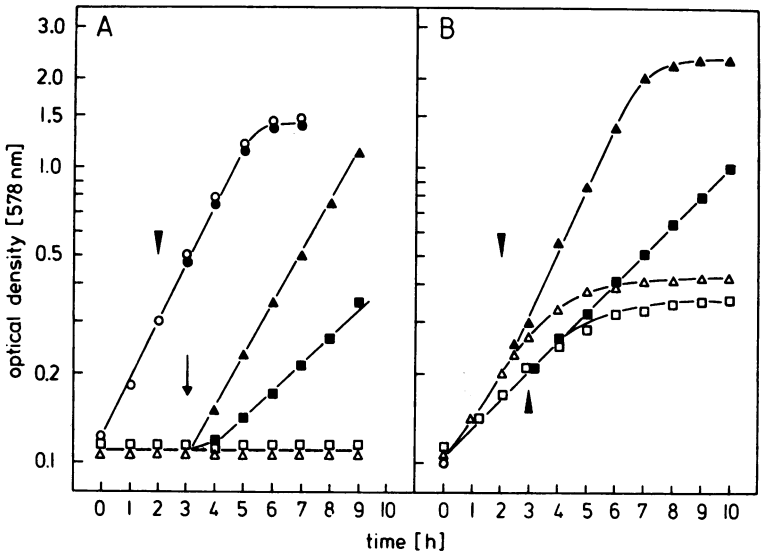

FIG. 1. Growth of different *ugp*$^+$ strains on G3P as the sole source of carbon or phosphate. A, Strain SH115 pregrown overnight in minimal medium A containing 0.2% glucose was divided into two fractions. One fraction of the culture was diluted into fresh medium containing 0.2% glucose as the carbon source (○). At the time indicated by an arrowhead the culture was divided, and to one part 0.2% G3P (final concentration) was added (●). The remaining fraction of the overnight culture was washed twice in Tris medium without phosphate and suspended in the same medium containing 60 μM P_i and 0.2% G3P (△). At the time indicated by an arrow the culture was divided, and to one part 0.2% (final concentration) glucose was added (▲). The square symbols represent growth curves of the *ugp*$^+$ *glpR* strain SH118 which was pregrown overnight in minimal medium containing 0.4% neutralized succinate as the carbon source. The cells were treated as described above for SH115 and suspended in Tris medium containing 0.2% G3P (□). At the time indicated by an arrow the culture was divided, and to one part 0.4% (final concentration) succinate was added (■). B, Cells of *ugp*$^+$ strains SH115 and SH118 were pregrown and pretreated as described above. Strain SH115 (*ugp*$^+$) was grown in Tris medium containing 60 μM P_i and 0.2% glucose as the carbon source (△). Strain SH118 (*ugp*$^+$ *glpR*) was grown in the same medium, except that 0.4% succinate was the carbon source (□). At the time points indicated by an arrowhead each culture was divided, and to one part of each 0.2% (final concentration) G3P was added (▲, ■).

entry. Therefore, the kinetic parameters of G3P uptake via the *ugp* and *glpT* systems were measured under comparable assay conditions. Whereas the *ugp*$^+$ strain SH115 could not grow on G3P, the *glpT*$^+$ strain 72 grew very well on this carbon source. The kinetic analyses of the two transport systems in comparable genetic background are shown in Fig. 2.

The extrapolation of the Lineweaver-Burk plots yields K_s values of 20 and 2 μM for the *glpT* and the *ugp* systems, respectively. The V_{max} values of the two systems are 50 pmol/s × 4.2 × 10^8 cells (corresponding to 1 ml of a cell suspension at an optimal density [578 nm] of 0.5) for the *glpT* system and 125 pmol/s × 4.2 × 10^8 cells for the *ugp* system. Thus, the *ugp* system has a higher affinity and a V_{max} that is even somewhat higher than in the *glpT* system.

Carbon derived from G3P and being transported by the *ugp* transport system is incorporated in phospholipids as well as in amino acids. Figure 3 shows the autoradiograph of a paper chromatogram with extracts of a *ugp*$^+$ strain that is in addition *glpT glpR* and *phoA*. This strain was growing with succinate as the carbon source and was exposed to 3 μM [*U*-^{14}C]G3P for different time intervals. The ^{14}C label in G3P quickly enters not only phospholipids but also TCA-precipitable material that is not phospholipid (Fig. 3). When these precipitates are hydrolyzed with 6 N HCl after extraction of phospholipids and analyzed the paper chromatography, radioactive label can be found even after 1 min in components behaving chromatographically like amino acids. As a control the same experiment was done with a *glpT*$^+$ *ugp*^{0} strain. Here G3P enters the cell exclusively via the *glpT* system; again the strain is *glpR phoA*, but is not derepressed for the *ugp* system. The same incorporation pattern as in a *ugp*$^+$ strain was obtained (data not shown). Therefore, it must be concluded that G3P entering the cell via the *ugp* system not only is funneled into phospholipids but must also have access to biosynthetic pathways of macromolecules. When the composition of the formed phospholipids was analyzed by thin-

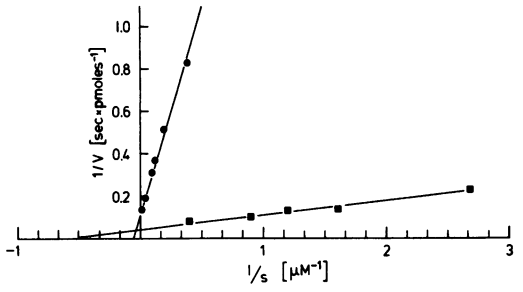

FIG. 2. Determination of apparent K_m and V_{max} of G3P uptake in constitutive ugp^+ and $glpT^+$ strains. Cells were grown in minimal medium A containing either glucose (strain SH115) or glycerol (strain 72) as the carbon source to an optical density (578 nm) of 0.5. Initial rates were measured by taking three samples within 20 s. The results are given as amount of [*U*-^{14}C]G3P taken up per 200 μl of cell suspension per s (optical density, 0.5) at room temperature. Symbols: ■, ugp^+ strain SH115; ●, $glpT^+$ strain 72.

layer chromatography all major phospholipids of *E. coli* were found in the usual relative composition (data not shown).

The ability of G3P to enter via the *ugp* transport system into the metabolic pool of the cell can also be demonstrated by the use of a mutant that has a G3P requirement due to an acyltransferase of reduced affinity for G3P (9). When this strain (BB26-36) was made *glpT*, G3P entering via the *ugp* system was able to satisfy the cells's G3P requirement (data not shown).

In addition, the competing effect of unlabeled glycerol for the incorporation of [^{14}C]G3P into TCA-precipitable material was tested in a ugp^+ *glpT* $glpK^+$ strain during growth on succinate. Inhibition by glycerol would occur after glycerol enters the cell and forms intracellular unlabeled G3P. Glycerol inhibited total incorporation of [*U*-^{14}C]G3P via *glpT* (strain 72) by 30% and via *ugp* (strain SH119) by 40%. The inhibition is the same for the incorporation of label in phospholipids as well as other TCA-precipitable material. Thus, G3P can enter the cell's metabolic pool after being transported by the *ugp* system.

Exposure to G3P without another carbon source inhibits uptake of G3P via the *ugp* transport system. In Figure 4, the ability of two strains to incorporate G3P into their cellular material is shown. For a strain that takes up G3P exclusively via the *glpT* system the incorporation of G3P is the same when G3P is the sole carbon source or when succinate is present as an additional carbon source. In contrast, when the *ugp* system is the only route of G3P entry the incorporation is dependent on the presence of an alternate carbon source. The better the carbon source, the better the G3P incorporation. Succinate increased G3P incorporation by a factor of 6 to 7; with glucose it becomes faster than the incorporation of G3P via the *glpT*-dependent system (Fig. 2).

Isolation of mutants defective in the *ugp*-dependent transport system. It has recently been reported that 3,4-dihydroxy-butyl-1-phosphonate (DHBP) is growth inhibitory in strains transporting G3P exclusively via the *glpT*- or *ugp*-dependent system (17). The data in Fig. 5 confirm this observation. In addition, the inhibition of G3P uptake (at 0.1 μM) via the *ugp*-dependent system was measured at increasing DHBP concentrations. The analog showed a half-maximal inhi-

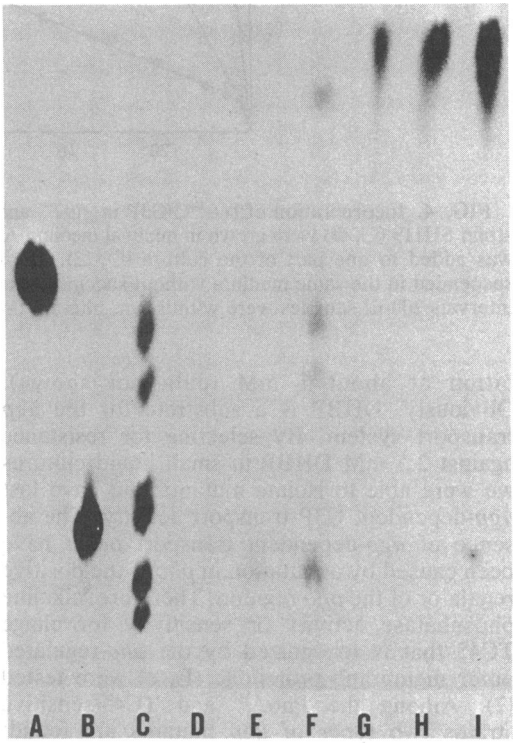

FIG. 3. Incorporation of [*U*-^{14}C]G3P in whole cells of a ugp^+ strain. Strain SH118 (*glpR phoA glpT* ugp^+) was grown in minimal medium A containing 0.4% succinate as the carbon source to an optical density (578 nm) of 0.5. It was labeled at 37°C with [*U*-^{14}C]G3P for different time intervals and precipitated with 10% TCA. Phospholipids were extracted, and the remaining precipitate was hydrolyzed with 6 N HCl. The resulting products were analyzed by paper chromatography and visualized by autoradiography. Lanes: A, glycerol; B, G3P; C, control extract of ugp^+ strain SH119 labeled with a mixture of different ^{14}C-amino acids; D through F, products of the hydrolysis of TCA-precipitable material after labeling for 30 s (D), 60 s (E), or 5 min (F) with [^{14}C]G3P; G through I, extracted phospholipids after labeling for 30 s (G), 60 s (H), or 5 min (I).

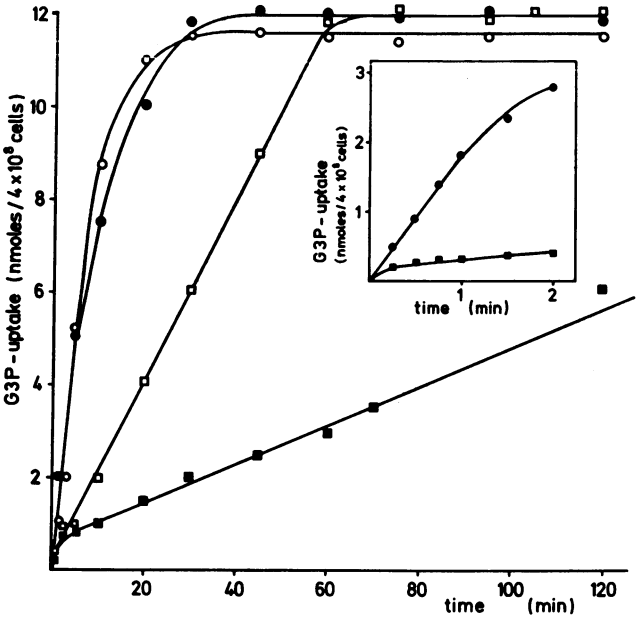

FIG. 4. Incorporation of [*U*-^{14}C]G3P in *glpT*$^+$ and *ugp*$^+$ strains. Cells of *glpT*$^+$ strain 72 (○, ●) and *ugp*$^+$ strain SH119 (□, ■) were grown in minimal medium A to an optical density (578 nm) of 0.5. [*U*-^{14}C]G3P (100 μM) was added to one part of the culture (○, □). The other part was washed once in minimal medium A and suspended in the same medium without succinate, and 100 μM [*U*-^{14}C]G3P was added (●, ■). At various time intervals 200-μl samples were withdrawn, filtered through membrane filters (Millipore) and counted.

bition at about 1 mM (data not shown). Obviously, DHBP is a substrate for the *ugp* transport system. By selecting for resistance against 2.5 mM DHBP in small liquid cultures we were able to isolate mutants that have lost *ugp*-dependent G3P transport activity. The absence of *ugp*-dependent transport might have been caused by a mutation in *phoB*, the positive regulator of the *pho* regulon. Therefore, alkaline phosphatase activity or sensitivity for phage TC45 that is recognized by the *pho*-regulated outer membrane protein Ic (E, e) were tested (7). Among the PhoA$^+$ and TC45-sensitive strains two types of *ugp* mutants are found: those that have lost the capacity to synthesize the G3P-binding protein, and those that still produce this protein normally (31a). We were also able to isolate the latter type of mutant (SH1200) after insertion of the tetracycline resistance transposon Tn*10* in *ugp*. The isolation of these two types of mutants suggests that the *ugp* system consists of more than one gene (31a). These mutations (*ugpA* defined by the *Tn*10 insertion and *ugpB* defined as the structural gene for the G3P-binding protein or a gene proximal to it on a multicistronic operon) are located at min 75 of the *E. coli* chromosome far away from the known location of other *pho* operons.

To confirm the role of the *ugp* system in providing cellular phosphate by entry of G3P we introduced (via Tn*10* transduction) a *ugp* mutation into the strain previously used for growth experiments. Figure 6 shows the ability of such a

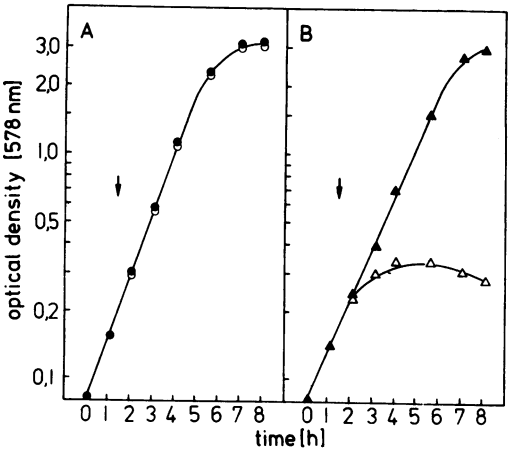

FIG. 5. Effect of DHBP on growth of *ugp*$^+$ strains. The culture medium consisted of minimal medium A (100 mM P_i) with 0.2% glucose as the sole carbon source. At the time points indicated by an arrow DHBP was added to a final concentration of 2.5 mM. A, Strain SH100 (*glpT ugp*^{0}): ●, untreated cells; ○, cells treated with DHBP. B, Strain SH101 (*glpT ugp*$^+$): ▲, untreated cells; △, cells treated with DHBP.

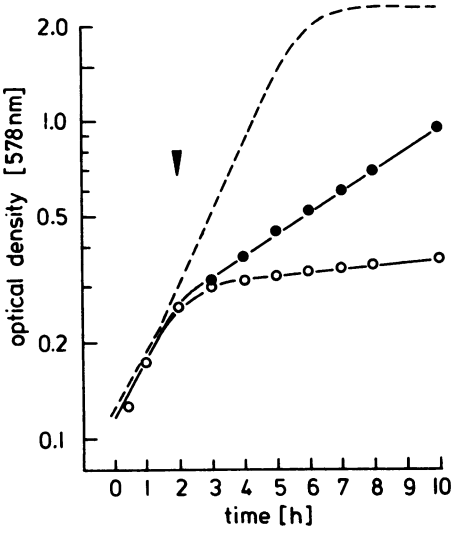

FIG. 6. G3P as the sole source of phosphate in a *ugp* strain. *ugp* strain SH117 was pregrown overnight in minimal medium A containing 0.2% glucose as the carbon source and was pretreated as described in the legend to Fig. 1. Cells were suspended in Tris medium containing 60 μM P_i and 0.2% glucose (○). At the time indicated by an arrowhead 0.2% (final concentration) G3P was added (●). The broken line shows the growth of the corresponding *ugp*$^+$ strain SH115 with G3P as the sole source of phosphate (Fig. 1A).

strain to use G3P as the sole source of phosphate. When grown on glucose the mutant can use P_i effectively as a phosphate source. When P_i was exhausted and G3P was the only phosphate source, the growth rate was reduced by a factor of 3 as compared with a *ugp*$^+$ strain growing on glucose and G3P as the only P_i source (Fig. 6). These results demonstrate that even in the absence of the *ugp* transport system G3P can be used, albeit poorly, as a phosphate source. The presence of periplasmic phosphatases other than alkaline phosphatase (30) may be responsible for the slow release of P_i from G3P in the periplasm.

The *ugp*-dependent G3P transport activity is not operating in membrane vesicles. To characterize further the *ugp*-dependent G3P transport system, the ability of membrane vesicles to take up G3P was investigated. Figure 7 is a comparison of the G3P transport activity in membrane vesicles of two strains, one *glpT*$^+$ *ugp*^{0} and the other *ugp*$^+$ *glpT*. Only the *glpT*$^+$ strain exhibited D-lactate-dependent transport activity in membrane vesicles. Vesicles of both strains transport proline normally (data not shown). This demonstrates that the *ugp*-dependent G3P transport does not operate in membrane vesicles, presumably due to the lack of the periplasmic G3P-binding protein that is removed during vesicle preparation. Consistent with this finding is the effect of cold osmotic shock on the *ugp*-dependent G3P transport activity (Fig. 7A).

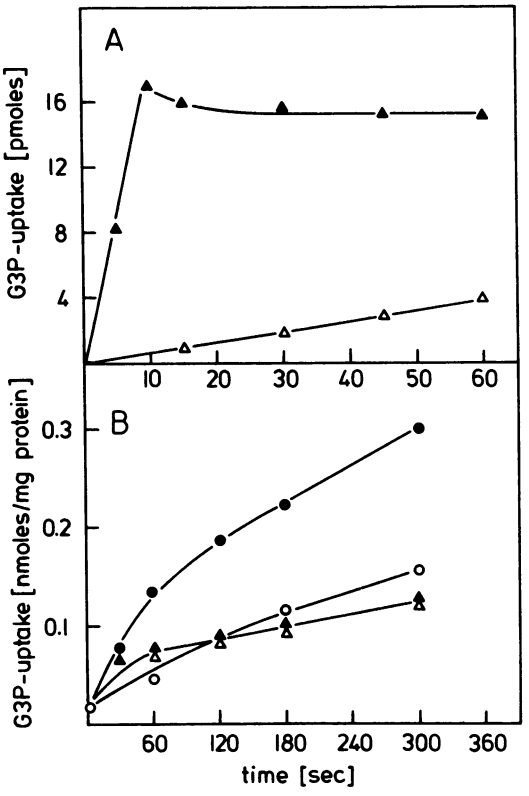

FIG. 7. G3P transport activity of the *ugp*$^+$- and *glpT*$^+$- dependent systems in whole cells, shocked cells, and membrane vesicles. A, Effect of cold osmotic shock on *ugp*$^+$-dependent G3P transport. Strain LA5301 was grown in minimal medium A with 0.2% glucose to an optical density (578 nm) of 0.5. A sample was removed, washed in 10 mM Tris-hydrochloride (pH 7.0)–150 mM sodium chloride and suspended in the same buffer to an optical density of 1.0, and transport was measured. The remaining culture was shocked by the method of Neu and Heppel (26) and suspended in 10 mM Tris-hydrochloride (pH 7.0)–150 mM sodium chloride to the original optical density (1.0), and transport activity was measured. Results are given as amounts taken up per 200 μl of cell culture (▲) or shocked cell suspension (△). B, Transport activity of the *glpT*$^+$- and *ugp*$^+$-dependent G3P transport systems in membrane vesicles. Cells were grown in minimal medium A with glucose (LA5301) and glycerol (TS100) as the carbon source to an optical density (578 nm) of 0.4. Membrane vesicles were prepared (19), and G3P transport activity was measured as described previously (6). Symbols: △, *ugp*$^+$ strain LA5301 without D-lactate; ▲, *ugp*$^+$ strain LA5301 plus 20 mM D-lactate; ○, *glpT*$^+$ strain TS100 without D-lactate; ●, *glpT*$^+$ strain TS100 plus 20 mM D-lactate.

DISCUSSION

E. coli contains two different transport systems for G3P that are geared for the effective utilization of carbon or phosphate. Thus, the *glpT*-dependent system is part of the *glp* regulon and is fully induced when the cells are growing on G3P (21). The transported G3P quickly enters the cells' metabolic pool. In contrast, the second system (*ugp* dependent) is under the control of the *pho* regulon and is present only when the cells are starved for phosphate or in mutants that are derepressed for the *pho* regulon (3). These derepressed cells cannot grow on G3P as the only carbon source despite the fact that they contain a highly active transport system for G3P. Possible explanations that would account for this phenomenon could be a V_{max} for G3P uptake too low for sustaining growth, an uninduced state of the catabolic G3P dehydrogenases, or the inability of G3P to reach the cytoplasm after being taken up by the transport system. As shown in this paper, none of these explanations was correct. When properly energized by glucose, uptake of G3P via the *ugp* system showed not only a lower K_m but also a higher V_{max} as compared with the *glpT* transport system; the latter is quite sufficient to supply G3P for growth. Also, an insufficient level of metabolizing enzymes cannot be the reason. Mutants were constructed that contained constitutive (*glpR*) levels of the necessary G3P dehydrogenases. They still showed the same inability to grow on G3P. Emphasis was put on the question of whether G3P is able to enter the cells' metabolic pool after being transported by the *ugp* system. This is clearly the case. When cells are growing on succinate, radioactively labeled G3P enters rapidly into phospholipids as well as into protein. The splitting of G3P in the periplasm and uptake of labeled glycerol were excluded in these experiments, since the strains lacked alkaline phosphatase and were defective in glycerol-kinase. On the other hand, in strains exhibiting a fully active kinase the addition of unlabeled glycerol gave an inhibition of G3P incorporation in a *ugp*$^+$ *glpT* that was similar to that of the corresponding *ugp*^{0} *glpT*$^+$ strain. Thus, G3P taken up by the *ugp* system is not funneled into a special pathway, but enters the common G3P pool. What is then the reason for the inability to grow on G3P as the only carbon source? G3P as a combined source of carbon and phosphate may only be used as the sole source of carbon as long as excess phosphate can be expelled. In the case of the *glpT*-dependent transport system this does not pose a problem, since P_i can be transported via the *glpT*-dependent G3P transport system. The *ugp*-dependent system does not recognize P_i. Therefore, an excess of P_i or another phosphorylated compound may accumulate internally and inhibit further uptake of G3P.

P_i produced from cytoplasmic G3P may be derived from the methylglyoxal pathway (12) after formation of dihydroxyacetone phosphate or after hydrolysis of phosphatidyl glycerolphosphate (29).

The uptake of G3P via the *ugp* system is most likely mediated by the periplasmic G3P-binding protein (2). Even though there is at present no direct evidence, several observations are consistant with a transport function of this protein: (i) it is coordinately regulated with *ugp* transport (4); (ii) it exhibits the same specificity pattern even though the K_d for G3P is 10-fold lower than the corresponding K_m of transport (2); (iii) transport-defective mutants can be isolated that lack the G3P-binding protein.

As with other classical binding protein dependent systems (32) the *ugp*-dependent G3P transport system consists of more than one component. This is clear from the isolation of *ugp* mutants that still synthesize the G3P-binding protein (31a).

In accordance with studies of other binding protein dependent systems (32) the *ugp* system is highly sensitive to the cold osmotic shock procedure (25) in whole cells and does not operate in membrane vesicles.

The existence of two types of transport systems for G3P and the comparison of their properties is analogous to the two types of P_i transport systems (31). Derepression by low P_i, high affinity, and the participation of a periplasmic binding protein are characteristic for one type (*ugp* and *pst*). The other type (*glpT* and *pit*) is not repressed by P_i, exhibits a lower substrate affinity, and is dependent on the proton motive force as energy coupling mechanism.

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