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Characteristics of a *ugp*-Encoded and *phoB*-Dependent Glycerophosphoryl Diester Phosphodiesterase Which Is Physically Dependent on the Ugp Transport System of *Escherichia coli*

PIUS BRZOSKA AND WINFRIED BOOS*

Department of Biology, University of Konstanz, 7750 Konstanz, Federal Republic of Germany

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The *ugp*-encoded transport system of *Escherichia coli* accumulates *sn*-glycerol-3-phosphate with high affinity; it is binding protein mediated and part of the *pho* regulon. Here, we report that glycerophosphoryl diesters (deacylated phospholipids) are also high-affinity substrates for the *ugp*-encoded system. The diesters are not taken up in an unaltered form but are hydrolyzed during transport to *sn*-glycerol-3-phosphate plus the corresponding alcohols. The enzyme responsible for this reaction is not essential for the translocation of *sn*-glycerol-3-phosphate or for the glycerophosphoryl diesters but can only hydrolyze diesters that are in the process of being transported. Diesters in the periplasm or in the cytoplasm were not recognized, and no enzymatic activity could be detected in cellular extracts. The enzyme is encoded by the last gene in the *ugp* operon, termed *ugpQ*. The product of the *ugpQ* gene, expressed in minicells, has an apparent molecular weight of 17,500. We present evidence that only one major *phoB*-dependent promoter controls all *ugp* genes.

sn-Glycerol-3-phosphate (G3P) can be utilized by *Escherichia coli* as the sole source of carbon (24) and of phosphate (36). Being an essential precursor for phospholipids, a sufficient concentration of G3P must be maintained by the cell (6, 33). In the absence of exogenous G3P, this essential intermediate is supplied by the reduction of dihydroxyacetone phosphate via an NADH-linked dehydrogenase, an enzyme that is tightly regulated by G3P (7). The major uptake system for G3P in the presence of P_i, which represses (43) the *pho* regulon, is the *glpT*-dependent transport system (22). G3P transport via the *glpT*-dependent transport system is mediated by exchange against intracellular P_i (1, 13). *glpT* is a member of the *glp* regulon, which consists of several genes organized into several operons, the products of which are responsible for uptake and catabolism of glycerol and G3P (24). The operon containing *glpT* harbors a second gene, *glpQ*, distal to *glpT*, that codes for a periplasmic glycerophosphoryl diester phosphodiesterase, an enzyme that hydrolyzes deacylated phospholipids to G3P and the corresponding alcohols (20). The *glp* regulon is induced by elevated levels of cytoplasmic G3P, the induction being controlled by a cytoplasmic repressor, the product of the *glpR* gene (23). The catabolic nature of the *glp* regulon is demonstrated by its dependence on the cAMP-catabolite gene activator protein regulatory circuit (24).

G3P is also a substrate for the hexose phosphate transport system (12). However, since this system is induced only when glucose-6-phosphate is present in the medium (17, 44), it does not play a major role in the utilization of G3P.

The *ugp*-encoded transport system (for a review, see reference 10), a third transport system recognizing G3P, is part of the *pho* regulon (for a review, see reference 43). The *ugp* genes are located at 75 min on the *E. coli* linkage map (40). At least three gene products are necessary for transport (38). Physiologically, the *ugp*-encoded system is geared for the utilization of phosphate from G3P. Mutants deleted for *glpT* and *glpQ*, constitutive for the *pho* regulon, and deleted for *phoA*, the structural gene for the periplasmic enzyme alkaline phosphatase, take up G3P only via the *ugp*-encoded system. However, when G3P is present as the sole source of carbon, these mutants cannot grow on G3P. Nevertheless, G3P can be incorporated into phospholipids and can be metabolized by G3P dehydrogenase if another carbon source is present. In this case, G3P can be utilized as the sole source of phosphate (36). At present, this phenomenon is not understood (10).

As a member of the *pho* regulon, the *ugp*-dependent transport system is expressed only when the concentration of P_i in the medium drops below 0.1 mM or in mutants that are constitutive for the *pho* regulon (30), such as *phoR* or *pst* mutants (39). The *ugp* region has been cloned, and some of the proteins encoded by the *ugp* genes have been identified (37, 38). The most prominent of these proteins is the periplasmic binding protein (3) that establishes the recognition site of the system. Therefore, the *ugp*-encoded system belongs to the family of binding protein-dependent transport systems, exhibiting membrane-associated proteins carrying ATP-binding sites, which are found only in gram-negative bacteria (2). Like other binding protein-dependent transport systems, the Ugp system recognizes G3P with a K_m that is on the order of micromolar concentrations (3, 4).

In contrary to previous reports (38), the *ugp* operon begins with the *ugpB* gene, which codes for the periplasmic binding protein. *ugpC* contains the ATP binding site consensus sequence. Furthermore, as elucidated by DNA sequencing, there is only one promoter proximal to *ugpB* that controls the entire *ugp* region. This promoter contains a classical consensus *phoB* binding site (Jan Tommassen, personal communication).

In the present work, we show that glycerophosphoryl diesters, the deacylation products of phospholipids, are substrates of the *ugp*-encoded transport system and are recognized to the same extent as G3P. Closely associated with the transport system is a specific glycerophosphoryl diester phosphodiesterase that recognizes the incoming diesters and hydrolyzes them to G3P plus the corresponding alcohols. In phosphodiesterase mutants, transport is not abolished and diesters are accumulated in an unmodified

* Corresponding author.

TABLE 1. List of strains, plasmids, and phages used in this study

Strain, plasmid, or phage	Relevant genotype	Reference(s) or source
Strains		
MC4100	F⁻ Δ(*argF-lac*)*U169 araD rpsL150 relA1 thiA deoC1 rpsL150*	11
TL45	MC4100, *glpR* Δ(*glpT-glpA*)*593 gyrA*	26
BB2636	HfrC *phoA8 glpD3 glpR2 glpK relA1 tonA22 plsB26 plsX* λ^r	6, 21
AC115	SH116, *glpR glpK*$^+$	Our collection
AC118	AC115, *ugpB706 zhf-721*::Tn*10*	Our collection
AC123	SH116, *glpT*$^+$ *glpK*$^+$	Our collection
DS410T	*minB ara lacY malA mtl xyl rpsL thi tonA azi* Δ(*glpT-glpA*)*593 gyrA*	38
SH116	HfrC, *glpK* Δ*glpT596 gyrA phoA8 phoT ilv*	36
SH120	TL45, *phoST*	39
SH131	SH120, *phoB23 proC*::Tn*5*	39
SH228	TL45, *ugpA704*::Tn*10*	38
Brz34	SH120, *phoA8 tsx*::Tn*10*	14
Brz35	Brz34, *glpD2*	14
Brz312	SH120, *glpT*$^+$ *gyrA*$^+$ *glpD2 phoA8 ugpA704*::Tn*10*	This study
Brz321	SH120, *phoA8 ugpA704*::Tn*10*	This study
Brz335	TL45, φ(*ugpQ-lacZ*)*1*(Hyb) (λ p*lac*Mu15)	This study
Brz336	TL45, φ(*ugpB-lacZ*)*1*(Hyb) (λ p *lac*Mu15)	This study
Brz337	TL45, φ(*ugp[A,C]-lacZ*)*1*(Hyb) (λ p *lac* Mu15)	This study
Brz338	Brz335, *phoST ilv*::Tn*10*	36
Brz339	Brz337, *phoST ilv*::Tn*10*	This study
Brz341	Brz336, *phoR*::Tn*10*	E. Bremer
Brz342	Brz337, *phoR*::Tn*10*	This study
Brz343	Brz335, *phoR*::Tn*10*	This study
Brz344	Brz335, *ugpA704*::Tn*10*	38
Brz346	Brz337, *ugpA704*::Tn*10*	This study
Brz347	Brz336, *phoST ilv*::Tn*10*	This study
Brz110	SH120, *glpD3 ugpQ5 zhf-721*::Tn*10*	This study
Brz111	Brz110, *glpD*$^+$	This study
Brz48	BB2636, Δ(*glpT-glpA*)*592 zei724*::Tn*10 gyrA phoR48* λ^s	22
Brz49	BB2636, Δ(*glpT-glpA*)*592 phoR48 gyrA*; spontaneous tetracycline-sensitive derivative of Brz48	This study
Brz495	Brz49, *ugpQ5*	This study
Brz422	Brz49, Δ(*argF-lac*)*U169 phoR*::Tn*10*	This study
Brz423	Brz422, φ(*ugpQ-lacZ*)*1*(Hyb) (λ p*lac*Mu15)	This study
Brz424	Brz422, φ(*ugpB-lacZ*)*1*(Hyb) (λ p *lac* Mu15)	This study
Brz425	Brz422, φ(*ugp[A, C]-lacZ*)*1* (Hyb) (λ p *lac*Mu15)	This study
Brz426	Brz495, *pcnB zae*::Tn*10*	25
Brz120	DS410T, *phoR*::Tn*10*	This study
Plasmids[a]		
pBR322	*bla tet*	8
pSH12	*ugpBACQ bla*	38
pSH9	*ugpACQ bla*	37
pBrz1	Δ*ugp bla*	This study
pBrz2	*ugpQ bla*	This study
pBrz3	*ugpQ bla*	This study
pGS31	*glpT glpQ tet*	15
Phages		
λ p *lac*Mu15	Mu cIts62 *ner*$^+$ *ara* Mu*S lacZ lacY*$^+$ *lacA imm* Aam1039 Kan^r	E. Bremer
λ pMu507	Mu cIts62 Mu *A*$^+$ Mu*B*$^+$ *S*am7	27

[a] The gene symbols in plasmids represent the wild-type alleles.

form. We present evidence that the enzyme activity is physically associated with the transport of the diesters. The enzyme is controlled by the same promoter that controls the other transport genes.

MATERIALS AND METHODS

Bacterial strains and growth media. All bacterial strains used are listed in Table 1. Growth media were prepared by the method of Miller (29). Phosphate-free G+L medium is described in reference 36. For growth of cells carrying plasmid derivatives of pBR322 (8), cells were grown in the presence of ampicillin (50 μg/ml). Strains were constructed by P1 *vir* transduction (41), as indicated in Table 1.

Cloning procedures. Plasmids were prepared and DNA fragments were subcloned essentially by the methods of Maniatis et al. (28). Restriction endonucleases and T4 DNA ligase were from Boehringer, Mannheim, Federal Republic of Germany, and New England BioLabs, Inc., Beverly, Mass.

Isolation of *ugpQ* mutants. *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine mutagenesis was done by the method of Miller

(29). Cells harboring β-galactosidase protein fusions with λ *plac*Mu15 in the presence of λ pMu507 helper bacteriophage (27) were isolated by the method of Bremer et al. (9).

Minicell preparation and labeling technique. Minicells were prepared and labeled as described previously (15), except that we incubated the cells in 0.5 ml of minimal medium A (MMA) containing 0.2% glucose and 25 μl of methionine assay medium (Difco). For labeling, we added 30 μCi of [^{35}S]methionine (>1,000 Ci/mmol; Amersham). The samples were analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (19) followed by autoradiography.

Preparation of [^{14}C]phosphatidylglycerol and [^{14}C]phosphatidylethanolamine labeled in the glycerol moiety. An overnight culture of strain AC123 was diluted 1:100 in 5 ml of G+L medium containing 1% Casamino Acids, 50 μg of thiamine per ml, 1 mM inorganic phosphate (pH 7), and 50 μCi of [^{14}C]G3P (144 mCi/mmol; New England Nuclear) and grown at 37°C. Since AC123 is defective in the aerobic G3P dehydrogenase (*glpD*), all of the radioactive G3P should be incorporated into phospholipids. Incorporation was monitored by filtering 20-μl samples through membrane filters (0.45-μm pore size; Millipore Corp.) followed by scintillation counting. After 20 h, 78% of the radioactive material was incorporated.

The culture was harvested and washed once with 3 ml of 100 mM Tris hydrochloride, pH 8. The cells were suspended in 2 ml of water; 5 ml of methanol and 2.5 ml of chloroform containing 1 mg of cardiolipin were added. The suspension was thoroughly vortexed and was subsequently incubated on ice for 3 h. After centrifugation at 4,000 × *g* for 30 min, the supernatant was transferred to another tube and 2.5 ml of chloroform and 2.5 ml of 2 M KCl were added. The resulting emulsion was agitated thoroughly. After centrifugation at 800 × *g* for 10 min, the chloroform phase was removed and dried at 33°C in a stream of air. The dried material was suspended in 1 ml of chloroform-methanol (2:1, vol/vol) and centrifuged. The supernatant was transferred to a silica gel thin-layer chromatography (TLC) plate (on aluminium foil; Merck) and developed in chloroform-methanol-acetic acid (65:25:8, vol/vol/vol). The bands were identified by autoradiography. In this system, the upper band is cardiolipin, the middle band is phosphatidylglycerol, and the lower band is phosphatidylethanolamine. The phosphatidylglycerol and phosphatidylethanolamine bands were cut out and eluted with chloroform-methanol (2:1, vol/vol). The eluates were evaporated at 37°C and washed in a solvent containing 1 ml of chloroform, 1 ml of 2 M KCl, and 1 ml of methanol. The yield with respect to the initially incorporated label was 46% phosphatidylethanolamine and 4.6% phosphatidylglycerol. Phosphatidyl[^{14}C]ethanolamine and phosphatidyl[^{3}H]choline were purchased from Amersham.

Preparation of radioactively labeled glycerophosphoryl diesters from phospholipids. Glycerophosphoryl ethanolamine (GPE), labeled in the glycerol or ethanolamine moiety, glycerophosphoryl glycerol (GPG), and glycerophosphoryl choline (GPC) were prepared by mild alkaline hydrolysis as described previously (18). Samples of the glycerophosphoryl diester solution were dried in a stream of air and suspended in water before use.

Preparation of [^{14}C]ethanolamine and [^{3}H]choline. A 100-μl amount of [^{14}C]ethanolamine-labeled GPE or of [^{3}H]choline-labeled GPC was incubated for 20 min with 100 μl of shock fluid (35 mg/ml) from the *glpQ*$^+$ strain DL291(pGS31). After addition of trichloroacetic acid (TCA) to a final concentration of 5% and incubation on ice, protein was removed by centrifugation. For binding or transport assays using these compounds, the TCA precipitation step was omitted.

TABLE 2. Growth of *plsB plsX* strains carrying different *ugp glpT* and *glpQ* alleles

Genotype		Growth[a] when supplemented with:		
Chromosome	Plasmid	Glycerol	G3P	GPC
ugp$^+$ *glpTQ*	*ugp* or *ugp*$^+$	+	+	+
ugp glpT$^+$ *glpQ*$^+$	*ugp* or *ugp*$^+$	+	+	+
ugp$^+$ *glpT*$^+$ *glpQ*$^+$	*ugp* or *ugp*$^+$	+	+	+
ugp glpT glpQ	*ugp*	+	−	−
	ugp$^+$	+	+	+
	ugpQ$^+$	+	−	−
ugpBAC$^+$ *ugpQ glpTQ*	*ugp*	+	+	−
	ugp$^+$	+	+	+
	ugpQ$^+$	+	+	+

[a] Growth of different strains on MMA containing 0.2% glucose as the carbon source and one of 1 mM glycerol, 1 mM G3P, or 1 mM GPC as the supplement.

Large-scale preparation of *E. coli* glycerophosphoryl diesters. A 200-g amount of *E. coli* cells (wet weight) was suspended in 750 ml of chloroform-methanol (1:2, vol/vol) overnight at 4°C. After adding 250 ml of chloroform and 250 ml of water, we added solid $MgCl_2$ until the phases separated. The chloroform phase was washed with 100 ml of 2 M $MgCl_2$, and the water phase was washed once with 100 ml of chloroform. After drying the combined chloroform phases, we prepared the glycerophosphoryl diesters by mild alkaline hydrolysis (18). We obtained 800 mg of glycerophosphoryl diesters by this procedure. The chemical nature of the glycerophosphoryl diesters was tested by complementation analysis of the G3P requirement of a *plsB plsX* strain (see below).

Complementation analyses. Strains Brz495 (*ugpQ ugpB*$^+$*A*$^+$*C*$^+$ *plsB plsX*) and Brz415 (*ugpA plsB plsX*) were transformed with different plasmids and tested for growth on MMA containing 0.2% glucose and either 1 mM G3P or 1 mM GPC. Brz495 does not grow on GPC, but it does grow on G3P as a phospholipid source. Strain Brz415 does not grow on either G3P or GPC. Thus, Brz495 harboring a plasmid containing only *ugpQ*$^+$ grows when supplemented with GPC, while Brz415 harboring a plasmid containing *ugpB*$^+$*A*$^+$*C*$^+$*Q*$^+$ can grow when supplemented with G3P or GPC (Table 2).

Determination of *ugpQ*- or *glpQ*-encoded phosphodiesterase activities. To analyze the hydrolysis products of the glycerophosphoryl diesters by the *glpQ*- or the *ugpQ*-encoded glycerophosphoryl diester phosphodiesterases, strains were grown in LB medium to logarithmic phase, harvested, and washed twice in MMA. They were suspended to an optical density at 578 nm of 5. A 10-μl amount of this suspension and 10 μl of substrate containing 0.005 μCi of ^{14}C were mixed and incubated for the time indicated in the figures. The substrate concentration was 12.8 μM for ethanolamine-labeled [^{14}C]GPE and 23 μM for [^{14}C]G3P. The cells were centrifuged in an Eppendorf centrifuge, and TCA was added to the supernatant to a final concentration of 5%. The cells were washed once in 0.1 ml of MMA and were resuspended in 10 μl of 5% TCA (soluble extract). After centrifugation, the supernatants were chromatographed on phosphoethyleneimine (PEI)-cellulose TLC plates (Merck) (condition A) or on silica gel (condition B). A mixture of 75 ml of *iso*-propanol, 5 g of TCA, 0.25 ml of 25% ammonia, and 25 ml of water was used as the solvent for PEI-cellulose plates

TABLE 3. Binding activity of the G3P-binding protein in different strains[a]

Shock fluid from strain:	G3P-binding protein	nmol of GPE bound/ mg of protein	nmol of G3P bound/ mg of protein[b]
SH120	+	11.3	ND
SH131	−	0.5	ND
AC118	−	0.4	0.4
AC115	+	5.5	7.3
SH228	+	0.9	ND
SH228(pSH12)	++	12.3	ND

[a] The binding of ethanolamine-labeled [^{14}C]GPE at 5.7 μM and of [^{14}C]G3P at 11.5 μM by a crude preparation of periplasmic proteins (protein concentration, 0.36 mg/ml) was measured. It should be noted that the strains are not isogenic in respect to their *pho* regulation. Owing to this fact, one cannot directly compare binding activities of strain SH228 (wild type) with that of the preparation from the other strains that express the *pho* regulon constitutively.
[b] ND, Not determined.

(condition A). For silica gel plates, *iso*-propanol–25% ammonia–water (70:20:10, vol/vol/vol) (condition B) was used. The dried TLC plates were exposed for 1 week to X-ray films. When choline-labeled [^{3}H]GPC was used as the substrate, cells were incubated with a 0.1 μM concentration of it. We used PEI-cellulose TLC plates with *iso*-propanol–25% ammonia–water (70:20:10, vol/vol/vol) as the solvent (condition C). The TLC plates were cut into 1-cm squares and counted in a scintillation counter by using toluene-based scintillation fluid (Lipoluma; J. T. Baker Chemical Co., Holland).

Transport assays. Transport assays were done basically by the method of Schweizer et al. (36). Cells were grown in LB medium to the logarithmic phase. After being harvested, they were washed twice in MMA and resuspended in the same medium to an optical density at 578 nm of 0.1 or 0.5. Radioactive substrate was added to 1 ml of cells at concentrations given in the legends to the figures. Aliquots (0.2 ml each) were filtered through Millipore filters (0.45-μm pore size) at different time intervals and washed with 5 ml of MMA. After the filters were dried, they were counted in a scintillation counter by using a toluene-based scintillation fluid. Data are given as picomoles taken up by 5×10^8 cells.

Preparation of shock fluids. Shock fluids were prepared by the method of Neu and Heppel (31). The growth medium for the strains listed in Table 3 was G+L–0.2% glucose–50 μM phosphate buffer. All other shock fluids were prepared after growth in LB medium.

Shock fluid obtained from 1,000 ml of culture was suspended in 0.5 ml of 10 mM Tris hydrochloride, pH 7, dialyzed overnight against the same buffer, and clarified by centrifugation. The yield was about 2 mg of protein.

Binding assays with shock fluids. Binding assays with shock fluids were performed by the method of Richarme and Kepes (35). A 10-μl amount of radioactive substrate (for the concentrations, see Table 3) and 10 μl of shock fluid were mixed. After incubation on ice for 10 min, the protein substrate complex was precipitated with 1 ml of saturated ammonium sulfate solution at 0°C and filtered through Millipore filters (0.45-μm pore size). After the filters were washed with 3 ml of saturated ammonium sulfate solution, they were dried and counted.

β-Galactosidase and alkaline phosphatase assays. β-Galactosidase and alkaline phosphatase reporter activities were assayed essentially by the method of Miller (29). Overnight cultures were grown in G+L medium with 2 mM P_i and 0.2% glucose as the carbon source. After being washed in the same medium, the culture was diluted five times in G+L–0.2% glucose with and without 2 mM P_i. After growth at 37°C for 3 h and addition of 2 mM P_i (final concentration) to the phosphate-free culture, 200 μl was mixed with 2.4 ml of Z-buffer (β-galactosidase) or 2.4 ml of 1 M Tris hydrochloride (pH 8) (alkaline phosphatase). Subsequently, 50 μl of 0.1% SDS and 50 μl chloroform were added. Reactions were started by the addition of 0.4 ml of 0.4% *o*-nitrophenyl galactoside (Serva) or *p*-nitrophenyl phosphate (Sigma), respectively. The reactions were stopped with 1 ml of 1 M Na_2CO_3 or 0.2 ml of 1 M K_2HPO_4. After centrifugation in a microcentrifuge, the optical density of the supernatant at 405 nm was measured. Enzymatic activity is given as micromoles of substrate hydrolyzed per minute per milligram of protein at room temperature. Molar extinction coefficients of 4,860 (*o*-nitrophenol) and 18,000 (*p*-nitrophenol) were used.

RESULTS

Glycerophosphoryl diesters are inhibitors of Ugp system-mediated G3P uptake. We observed that the presence of 0.2% yeast extract (Difco) inhibited Ugp system-mediated uptake of 0.1 μM G3P by 95%. This inhibitor is not G3P since treatment of yeast extract with alkaline phosphatase did not reduce this inhibitory capability. Furthermore, we could not detect any G3P in yeast extract with an enzyme assay using G3P dehydrogenase.

However, treatment of yeast extract with the *glpQ*-encoded periplasmic glycerophosphoryl diester phosphodiesterase in combination with alkaline phosphatase abolished the inhibitory effect. This finding indicated that yeast extract contains deacylated products of phospholipids and that these glycerophosphoryl diesters were inhibiting Ugp system-mediated uptake of G3P. Indeed, authentic GPE and GPC at 10 μM inhibited the Ugp system-mediated uptake of 0.13 μM G3P by 95% and 80%, respectively.

Crude preparations of periplasmic proteins of strains expressing the Ugp system but lacking *glpQ* were able to bind ethanolamine-labeled [^{14}C]GPE (Table 3). (We used *glpT glpQ* mutants throughout the entire study to avoid any superimposition of the effects of these systems on the *ugp*-encoded transport system.) The same preparations bound G3P but not ethanolamine, and binding of ethanolamine-labeled GPE was inhibited by G3P, confirming the assumption of GPE and G3P being recognized by the same binding protein (data not shown). These observations demonstrated that ethanolamine-labeled GPE was specifically bound by the Ugp periplasmic binding protein.

To test whether glycerophosphoryl diesters are actually transported by the Ugp system, we used ethanolamine-labeled [^{14}C]GPE and glycerol-labeled [^{14}C]GPE as well as choline-labeled [^{3}H]GPC as substrates in the transport assay. Figure 1 shows that the latter two substrates are taken up, while the first of these three is not.

The curious differences between ethanolamine-labeled [^{14}C]GPE and glycerol-labeled [^{14}C]GPE as transport substrates could be explained in two ways. (i) The glycerophosphoryl diesters are hydrolyzed prior to transport, and only the G3P moiety of the molecule is taken up. Since label derived from choline-labeled [^{3}H]GPC is accumulated, this explanation is not plausible. One would expect in this case that the choline moiety is not taken up. (ii) The glycerophosphoryl diesters are hydrolyzed during or after their transport. Ethanolamine, the hydrolysis product of ethanolamine-labeled [^{14}C]GPE, is membrane permeable; it quickly diffuses out of the cytoplasm. Choline, the hydrolysis product of choline-labeled [^{3}H]GPC, is much less membrane

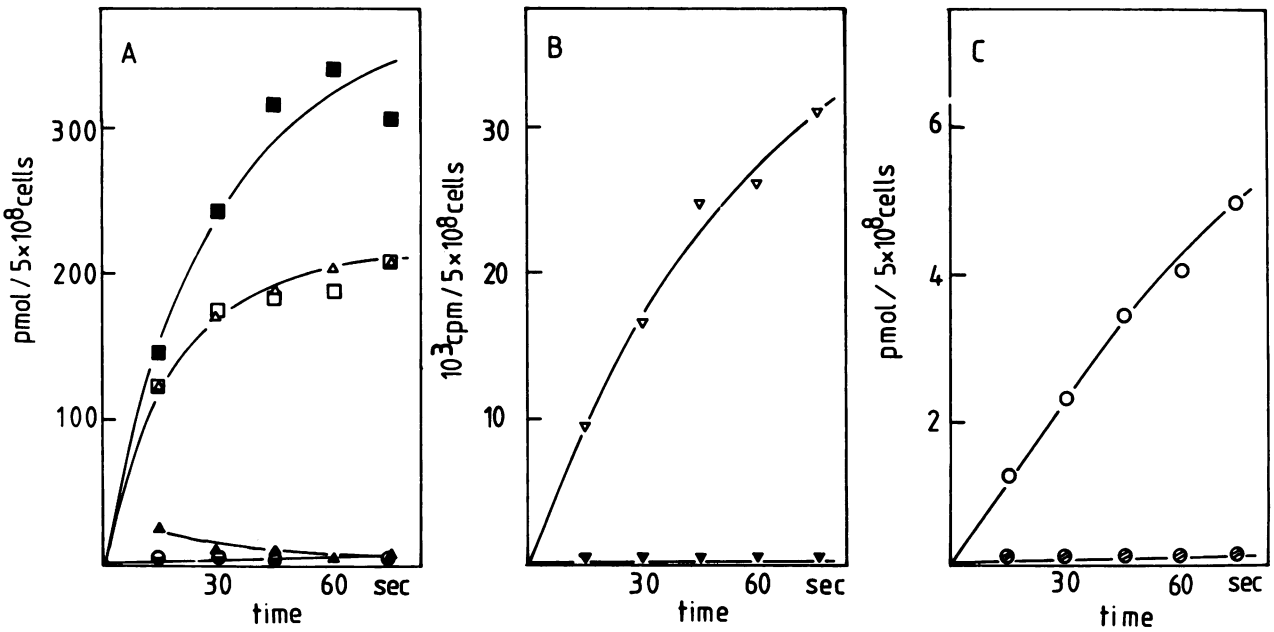

FIG. 1. Transport of G3P, GPE, and GPC through the Ugp system. (A) Triangles indicate transport of [^{14}C]G3P (0.13 μM) (△) and transport of ethanolamine-labeled [^{14}C]GPE (0.18 μM) (▲) by strain Brz35 (*ugp*$^+$ *glpT glpQ*). (○) (control), Transport of [^{14}C]G3P and ethanolamine-labeled [^{14}C]GPE at the same concentrations by strain Brz321 (*ugp glpT glpQ*). Squares indicate transport of [^{14}C]G3P (0.13 μM) (□) and of ethanolamine-labeled [^{14}C]GPE (0.18 μM) (■) by strain Brz110 (*ugpB*$^+$ *A*$^+$ *C*$^+$ *ugpQ glpT glpQ*). (B) Transport of glycerol-labeled [^{14}C]GPE in strain Brz35 (▽) and Brz321 (▼). For technical reasons, the specific activity of the substrate could not be determined. (C) Transport of 6.7 nM choline-labeled [^{3}H]GPC (○) and 6.7 nM [^{3}H]choline (○) by strain Brz35.

permeable and will accumulate. As will be explained below, the second explanation is the correct one.

A glycerophosphoryl diester phosphodiesterase is associated with the Ugp transport system. To identify the hydrolysis products that result from the uptake of glycerophosphoryl diesters, we analyzed by TLC the supernatant as well as the cellular extracts after incubating cells with [^{14}C]labeled G3P and glycerophosphoryl diesters labeled in different portions of the molecule. We used a mutant that was *glpT glpQ* and that thus could neither transport these substrates independently of Ugp nor hydrolyze the diesters by the GlpQ system. The strain was also unable to metabolize G3P because of defects in the aerobic G3P dehydrogenase (*glpD*) and alkaline phosphatase (*phoA*). The strain was still able to incorporate G3P into phospholipids. However, the cellular extracts were obtained by treatment of the cells with 5% TCA, and lipids were not detected by TLC because of their insolubility in TCA. Consequently, we could detect the first product of transport through the Ugp system.

The analysis is shown in Fig. 2. When G3P was used as the substrate, it disappeared from the supernatant and accumulated in the cytoplasm without chemical alterations (Fig. 2A). With ethanolamine-labeled GPE as the substrate, the label also disappeared from the medium, whereas ethanolamine was formed and appeared in the supernatant (Fig. 2B). No ethanolamine-labeled GPE was observed in the cellular extracts; we repeated this experiment several times under various conditions of time and substrate concentration but could never detect it inside the cell. With glycerol-labeled GPE as the substrate, label disappeared from the medium and glycerol-labeled G3P accumulated inside the cell. Again, no free glycerol-labeled GPE was found inside the cell (Fig. 2C).

A similar phenomenon was observed with glycerol-labeled glycerophosphoryl glycerol (GPG) as the substrate. As the free substrate disappeared from the medium, glycerol-labeled G3P, but not glycerol-labeled GPG, accumulated inside the cell, whereas glycerol appeared outside.

By using choline-labeled [^{3}H]GPC as the substrate, choline released during transport into the cytoplasm diffuses back into the medium only slowly. Of 1.5 pmol of choline accumulated after 30 s in the cytoplasm by 5×10^7 cells, only 0.13 pmol was released into the medium. Again, no accumulation of chemically unaltered choline-labeled [^{3}H]GPC was detectable in the cytoplasm.

These results suggested that the phosphodiesters undergo hydrolysis during transport. Since this strain does not actively transport choline under these conditions (Fig. 1C), it must be released in the cytoplasm and, in contrast to ethanolamine or glycerol, the cleavage products of GPE and GPG, choline can only diffuse slowly through the membrane into the medium. The hydrolytic activity is *phoB*- and *ugp*-dependent since *phoB* or *ugp* mutant strains do not hydrolyze phosphodiesters (data not shown). In analogy to the *glpQ*-encoded periplasmic phosphodiesterase, we termed the gene encoding the Ugp system-dependent phosphodiesterase *ugpQ*.

The appearance of glycerol in the supernatant when glycerol-labeled GPE was used as the substrate (Fig. 2C) is at present not understood. Glycerol cannot have been released by the hydrolysis of G3P since G3P itself is stable (Fig. 2A). Nor is it possible that the products of UgpQ -mediated hydrolysis are glycerol and phosphorylethanolamine, since G3P, not phosphorylethanolamine, emerged as the product (Fig. 2C) when ethanolamine-labeled GPE was used as the substrate (Fig. 2B).

It is very unlikely that the *ugpQ*-encoded enzyme is localized in the periplasm. This becomes clear when the activity profile of the *glpQ*-encoded periplasmic glycerophosphoryl diester phosphodiesterase is compared with that

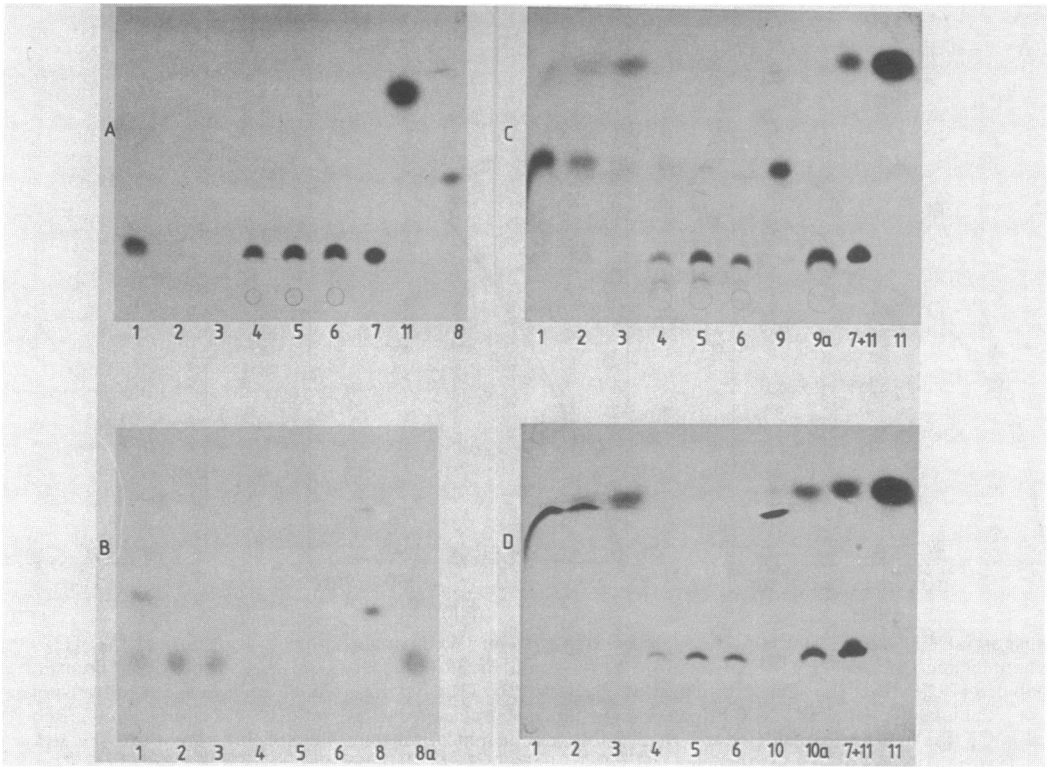

FIG. 2. Hydrolysis of different glycerophosphoryl diesters. Strain Brz35 (*ugp*$^+$) was incubated with [^{14}C]G3P (A), ethanolamine-labeled [^{14}C]GPE (B), glycerol-labeled [^{14}C]GPE (C), and glycerol-labeled [^{14}C]GPG (D). Supernatants of the cells and cytoplasmic fractions were chromatographed on TLC plates (condition B) and autoradiographed. Lanes: 1, 2, and 3, supernatant of cells after 0.5, 2, and 5 min, respectively; 4, 5, and 6, cytoplasmic fractions of cells after 0.5, 2, and 5 min, respectively. Controls: G3P (lane 7), ethanolamine-labeled GPE (lane 8), ethanolamine (lane 8a), glycerol-labeled GPE (lane 9), G3P derived from glycerol-labeled GPE (lane 9a), glycerol-labeled GPG (lane 10), G3P and glycerol derived from glycerol-labeled GPG (lane 10a), and glycerol (lane 11). The compound slightly below GPE in panel C (lanes 4, 5, and 6) is perhaps lysophospholipid. It also occurs in the case of GPG (panel D, lanes 4, 5, and 6). We cannot explain the appearance of glycerol in the supernatant in the experiment shown in panel C.

of the Ugp system-dependent enzyme. A *ugp glpT*$^+$ *glpQ*$^+$ mutant (Fig. 3A) and a *ugp*$^+$ *glpT glpQ* mutant (Fig. 3B) were incubated with glycerol-labeled GPE, and the supernatants were analyzed by TLC after 1 and 3 min. The first strain released G3P into the supernatant owing to the action of the periplasmic phosphodiesterase. In contrast, the second strain did not, even though GPE was effectively hydrolyzed and ethanolamine was released into the medium (compared with the results shown in Fig. 2B). The activity of the Ugp system is proven by the disappearance of glycerol-labeled GPE from the supernatant (Fig. 3B).

Consistent with this observation are the absence of a glycerophosphoryl diester phosphodiesterase activity in preparations of periplasmic proteins of a *ugp*$^+$ *glpT glpQ* strain (data not shown) and the accumulation of the hydrolysis products of choline-labeled GPC in the cytoplasm (Fig. 1).

Isolation of a mutant defective in the Ugp system-associated glycerophosphoryl diester phosphodiesterase. To characterize the enzyme further, we isolated a bacterium with a mutation in the *ugpQ* gene on the basis of the following rationale. A *plsB plsX* strain is unable to grow without G3P or glycerol owing to a K_m defect in the G3P acyltransferase, the enzyme which is responsible for the first step in phospholipid biosynthesis (6, 21). Table 2 shows that glycerophosphoryl diesters are able to supplement this requirement also. This can be due to either the action of the *glpQ*-encoded phosphodiesterase, which hydrolyzes the substrate to G3P,

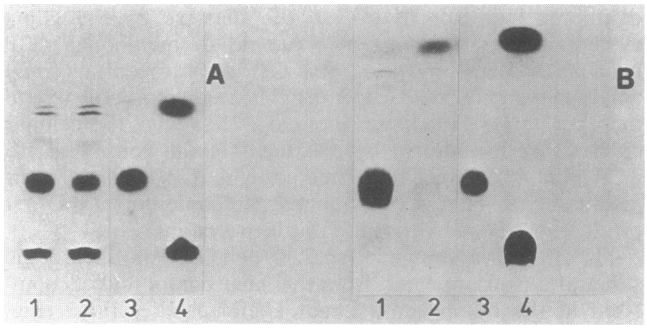

FIG. 3. Difference in the function of UgpQ- and GlpQ-dependent glycerophosphoryl diester phosphodiesterase activities. Strain Brz312 (*ugp glpT*$^+$ *glpQ*$^+$) and strain Brz35 (*ugp*$^+$ *glpT glpQ*) were incubated with glycerol-labeled [^{14}C]GPE for 1 and 3 min. The supernatants were analyzed by TLC (condition B), followed by autoradiography for the following times: Brz312, 1 min (panel A, lane 1); Brz312, 3 min (panel A, lane 2); Brz35, 1 min (panel B, lane 1); Brz35, 3 min (panel B, lane 2). Controls: glycerol-labeled GPE (panel A, lane 3, and panel B, lane 3); G3P and glycerol, lower and upper spot, respectively (panel A, lane 4, and panel B, lane 4). The appearance of glycerol in panel B, lane 2, is not understood (compare also Fig. 2).

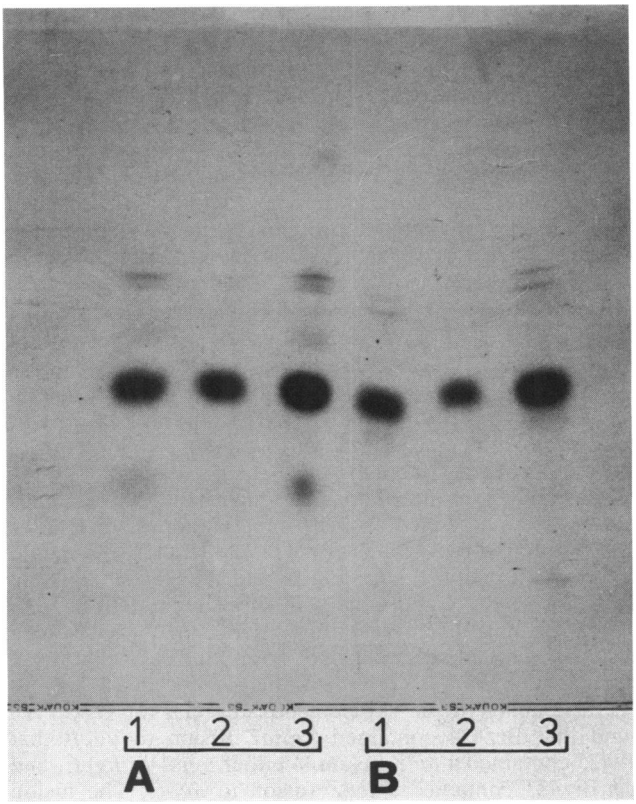

FIG. 4. *ugpQ* strains accumulate, but do not hydrolyze, GPE. Strain Brz110 (*ugpQ*) was incubated with glycerol-labeled [^{14}C]GPE or with ethanolamine-labeled [^{14}C]GPE for 2 min. Supernatant and cytoplasmic fractions of cells were analyzed by TLC (condition B) followed by autoradiography. Lanes: panel A, lane 1, glycerol-labeled [^{14}C]GPE, supernatant; panel A, lane 2, glycerol-labeled [^{14}C]GPE, cytoplasmic fraction; panel B, lane 1, ethanolamine-labeled [^{14}C]GPE, supernatant; panel B, lane 2, ethanolamine-labeled [^{14}C]GPE, cytoplasmic fraction; controls, glycerol-labeled [^{14}C]GPE (panel A, lane 3) and ethanolamine-labeled [^{14}C]GPE (panel B, lane 3). The corresponding analysis with the *ugp*$^+$ strain, Brz35, is shown in Fig. 2.

which is then accumulated by GlpT$^+$ cells, or by the accumulation of the intact diesters by Ugp$^+$ cells, followed by *ugpQ*-mediated hydrolysis. A *glpT glpQ* mutant expressing *ugp* but deficient in the *ugpQ*-encoded phosphodiesterase should be able to grow on G3P but not on glycerophosphoryl diesters as a phospholipid precursor.

After *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine mutagenesis of a *plsB plsX glpT glpQ* strain, which expresses the *ugp*-mediated system constitutively, colonies were grown on G3P-containing medium and replica plated onto plates containing glycerophosphoryl diesters as the sole source of phospholipid precursor. Of 12,000 colonies, 3 were found that could be complemented by G3P but not by glycerophosphoryl diesters. Two of these colonies exhibited defects in Ugp transport system activity and were discarded. The remaining mutant (Brz495) exhibited normal G3P uptake and contained wild-type levels of *ugp*-encoded periplasmic binding protein. However, GPE transport (Fig. 1A) was strikingly different from that of the wild type; radioactivity associated with ethanolamine-labeled GPE was accumulated by the mutant. In addition, TLC analysis revealed that the mutant cells (Fig. 4), in contrast to the wild-type cells (Fig. 2), accumulated chemically unaltered glycerol-labeled GPE or ethanolamine-labeled GPE after incubation with the diester for 2 min.

The mutation causing this phenotype was closely linked to or located within the *ugp* region; the *zhf-721*::Tn*10* insertion, which is 64% cotransducible with *ugp* (40), was introduced into the mutant. Of 100 tetracycline-resistant transductants, 27 lost the mutant phenotype. In addition, when the mutation was cotransduced with *zhf-721*::Tn*10*, 30 of 50 tetracycline-resistant transductants had acquired the *ugpQ* mutation.

Subcloning of the *ugpQ* gene. The *ugp* region previously had been cloned in pBR322 on a 7-kilobase DNA fragment (37, 38). When this plasmid, pSH12, was introduced into the *ugpQ* strain Brz495, the defect in the Ugp system-associated glycerophosphoryl diester phosphodiesterase activity was fully complemented. Also, plasmid pSH9 (Fig. 5), which contains part of *ugp*, was able to complement. Whereas pSH12 carries the major *phoB*-dependent promoter controlling the *ugp* region, *ugp* genes on pSH9 are controlled by either a second weak *phoB*-independent promoter or a vector-encoded one (38; unpublished results). pSH9 lacks the *ugpB*-encoded binding protein, whereas all other *ugp* genes are intact. Deletions made in plasmid pSH9 yielded several new smaller plasmids shown in Fig. 5. When tested for their ability to complement the UgpQ$^-$ phenotype in Brz495, it was found that the complementing activity was encoded by a 1.3-kilobase *Eco*RV-*Pvu*II fragment at the distal end of the *ugp* operon. This fragment, containing *ugpQ*, could not be expressed by the major *ugp* promoter since the upstream *ugp* sequences had been removed by the

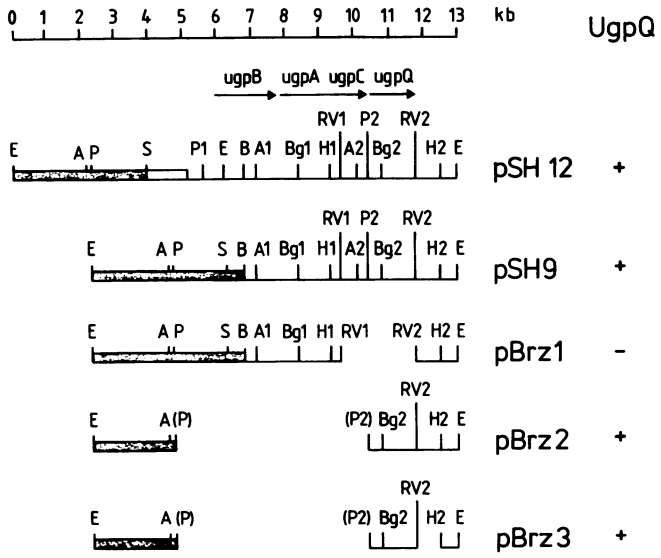

FIG. 5. Plasmids containing all or part of the *ugp* operon. pSH12 and pSH9 are described in references 37 and 38. pBrz1 is an *Eco*RV deletion of pSH9. pBrz2 is a *Pvu*II deletion of pSH9. The unique *Pvu*II site in this plasmid was lost during construction. pBrz3 is an *Hpa*I/*Eco*RV deletion of pBrz2. Abbreviations: E, *Eco*RI; S, *Sal*I; P, *Pvu*II; B, *Bam*HI; Bg, *Bgl*II; RV, *Eco*RV; H, *Hpa*I; A, *Acc*I. The original insert contains two *Pvu*II sites, two *Acc*I sites, two *Eco*RV sites, two *Hpa*I sites, and two *Bgl*II sites, marked 1 and 2. The arrows at the top of the figure are defined by complementation groups. The open segment indicates λ DNA (38); the stippled segments indicate vector DNA. This restriction map does not correspond entirely to the previously published map (38). It has been corrected on the basis of the newly derived DNA sequence data (Jan Tommassen, personal communication).

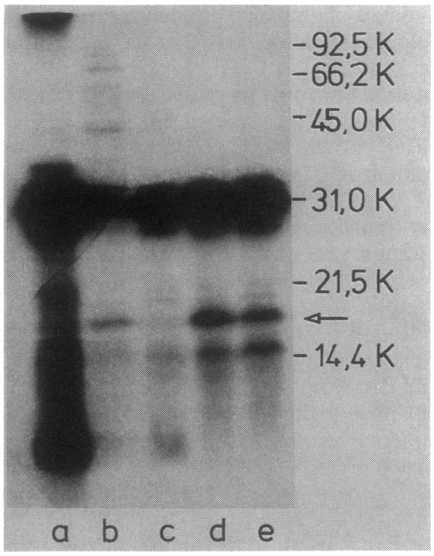

FIG. 6. Expression of the plasmids containing different *ugp* genes by [^{35}S]methionine-labeled minicells. The minicell-producing strain, Brz120, carrying plasmids pBr322 (lane a), pSH9 (lane b), pBrz1 (lane c), or pBrz3 (lanes d and e) was labeled with [^{35}S]methionine. Solubilized proteins were electrophoresed on a 12% SDS polyacrylamide gel and autoradiographed. The arrow indicates the position of the *ugpQ* gene product. The *ugpA* gene product is not identifiable in minicells. The *ugpC* gene product has a molecular weight of 40,000 (38).

subcloning (see below). However, all Tn*5* insertions that had been isolated in a Ugp$^+$ plasmid (37) resulting in a Ugp$^-$ phenotype were still able to complement the *ugpQ* mutation in Brz495 (data not shown). Therefore, either *ugpQ* is expressed in these plasmids by vector- or Tn*5*-associated promoter activity or *ugpQ* is independent of the major *ugp* promoter.

The smallest DNA fragment containing *ugpQ* encodes a protein of molecular weight 17,500. In order to identify the Ugp system-associated glycerophosphoryl diester phosphodiesterase, we transformed the plasmids shown in Fig. 5 into the minicell-producing strain Brz120, isolated the minicells, and labeled plasmid-encoded proteins with [^{35}S]methionine. SDS-solubilized proteins were separated by polyacrylamide gel electrophoresis and visualized by autoradiography. Figure 6 shows that a protein of molecular weight 17,500 is identical to the enzyme. The protein was produced by all plasmids that complement the *ugpQ* mutation but was not encoded by pBrz1, the only plasmid unable to complement the *ugpQ* mutation. This result is consistent with the finding of Schweizer and Boos (38), who identified on plasmid pSH12 six insert-encoded proteins. One of them had a molecular weight of 18,000 and was most likely identical to the *ugpQ* product.

Regulation of *ugpQ* and promoter analysis. To study the regulation of *ugpQ*, we isolated *lacZ* protein fusions in *ugpQ*. We used the same strategy of mutant screening that had enabled us to isolate Brz495 cells with the *ugpQ* mutation. For this purpose, strain Brz422 (Δ*lac plsB plsX*) was mutagenized with phase λ p*lac*Mu15 and Lac$^+$ colonies were selected on plates containing 0.2% lactose and 0.05% glycerol to supplement the *plsB plsX* requirement. About 40,000 colonies were screened on plates containing either G3P or GPC. We found two strains (Brz424 and Brz425) that could not grow with supplementation by either G3P or GPC and one (Brz423) that could grow with supplementation by G3P but not with supplementation by GPC. We transformed these fusion strains with plasmids pSH9, pSH12, and pBrz3, which contain various portions of the *ugp* operon (Fig. 5), to test their ability to be supplemented by G3P or GPC. We found that Brz424 contained a *lacZ* fusion to *ugpB* that Brz425 contained a *lacZ* fusion to either *ugpA* or *ugpC*, and that Brz423 contained a *lacZ* fusion to *ugpQ*. The fusion strain could be transduced with phage P1 into the starting strain Brz422 by selecting for Lac$^+$ and yielded the same phenotype. P1-mediated transductions revealed that the cotransduction frequency of the *ugpQ-lacZ ugp*[*A,C*]*-lacZ*, and *ugpB-lacZ* fusions with the *ugpA704*::Tn*10* allele were 79, 90, and >99%, respectively (200 transductants tested). This finding confirms that they map in the *ugp* region.

Table 4 shows the β-galactosidase activity of the *ugp-lacZ* fusion proteins in strains with various *pho* regulatory backgrounds. Also shown is chromosomal alkaline phosphatase activity as an internal control to show the regulatory state of the *pho* regulon. All three fusion proteins were coregulated with alkaline phosphatase. A *phoR* or a *phoT* mutant did express the fusion protein and alkaline phosphatase consti-

TABLE 4. β-Galactosidase activity of strains carrying different *ugp-lacZ* fusions in different *pho* regulatory backgrounds

Strain	Medium[a]	β-Galactosidase activity (U/mg of protein), 10^3	Alkaline phosphatase activity (U/mg of protein), 10^3
Brz335 (*ugpQ-lacZ*)	l	36	79
	h	7	6
Brz336 (*ugpB-lacZ*)	l	81	79
	h	7	4
Brz337 (*ugp*[*A, C*]*-lacZ*)	l	136	177
	h	9	10
Brz343 (*ugpQ-lacZ phoR*)	h	54	152
Brz341 (*ugpB-lacZ phoR*)	h	47	210
Brz342 (*ugp*[*A, C*]*-lacZ phoR*)	h	244	245
Brz338 (*ugpQ-lacZ phoS*)	h	244	588
Brz347 (*ugpB-lacZ phoS*)	h	854	1,248
Brz339 (*ugp*[*A, C*]*-lacZ phoS*)	h	1,310	1,389
Brz344 (*ugpQ-lacZ ugpA*::Tn*10*)	l	5	93
	h	2	5
Brz346 (*ugp*[*A, C*]*-lacZ ugpA*::Tn*10*)	l	7	104
	h	1	3

[a] The strains were incubated in 2 mM phosphate medium (h) or in medium without phosphate (l).

tutively. Furthermore, when derepressed by limited P_i concentrations, the fusion proteins were induced by a factor of 15 to 30 in a manner similar to induction by alkaline phosphatase. The *ugpA704*::Tn*10* insertion rendered the *ugpQ*- and *ugp*[*A,C*]-*lacZ* fusions no longer derepressible, whereas alkaline phosphatase expression was similar to that of the wild type. This indicates that the *ugpA704*::Tn*10* allele is polar on *ugpQ-lacZ* and also on *ugp*[*A,C*]-*lacZ*. These results show that all fusions, including *ugpQ-lacZ*, were under *pho* regulatory control and that *ugpQ* is located distal to *ugpAC* in the same operon. These and previous data (37) establish the order of the *ugp* genes as *ugpBACQ*.

Cytoplasmic glycerophosphoryl diesters are not substrates of the *ugpQ*-encoded enzyme. So far, the Ugp system-associated glycerophosphoryl diester phosphodiesterase activity could be observed only in intact cells exhibiting Ugp system-dependent transport. Attempts to detect this activity in cellular extracts, in membranes, or in detergent-solubilized cellular extracts were unsuccessful. However, all cellular extracts, regardless of their *ugp* or *pho* genotype, exhibited slow hydrolysis of GPE to G3P plus ethanolamine for a $ugpQ^+$ and a *ugpQ* strain. This activity was about 100-fold lower than the activity exhibited by intact $ugpQ^+$ cells. By using a *cpdB* mutant strain, we showed that this phosphodiesterase activity is not due to the periplasmic *cpdB*-encoded cyclic nucleotide phosphodiesterase (data not shown) (5).

Since we were unable to determine the *ugpQ*-encoded enzyme activity in cellular extracts, we wanted to test whether the phosphodiesterase activity is dependent on Ugp system-mediated transport.

To address this question, we considered the following. GPE can reach the cytoplasm only through the *ugp*-dependent transport system. Glycerophosphoryl diesters are not substrates of the glpT-encoded G3P transport system. If we could lower *ugpQ* expression without lowering the expression of the other *ugp* genes, one would expect that the rate of GPE transport would become faster than its rate of hydrolysis. Consequently, cells would accumulate intact GPE, in contrast to the wild type. If GPE being transported and GPE free in the cytoplasm were equally recognized by the enzyme, accumulated GPE would be hydrolyzed. If, on the other hand, only GPE in the process of being transported is recognized as the substrate, GPE would not be hydrolyzed after it had reached the cytoplasm. Such a situation is realized in strain Brz426(pBrz3). This strain is derepressed for the chromosomal *ugp* genes but carries a mutation in *ugpQ*. It was transformed with the plasmid pBrz3 containing the $ugpQ^+$ gene that is only weakly expressed and not under *pho* control. In addition, expression is further reduced since the copy number of pBrz3 is lowered by the chromosomal *pcnB* mutation (25).

Transport assays with ethanolamine-labeled GPE as the substrate revealed that it is partially accumulated in strain Brz426(pBrz3), in contrast to Brz49, its $ugpQ^+$ parent strain (data not shown). This indicates that the *ugpQ*-encoded enzyme is indeed the rate-limiting step in the whole process of transport and hydrolysis.

Figure 7 shows the fate of GPE in both strains analyzed by TLC. Cells were allowed to accumulate ethanolamine-labeled GPE for 20 min and to hydrolyze their accumulated material during a time span of 10 min after resuspension in substrate-free medium. The accumulated substrate in the mutant strain remained stable. The fast appearance of ethanolamine in the medium during initial uptake of GPE indicates that the *ugpQ*-encoded enzyme is active. From this experiment, it is clear that only transported GPE, not cytoplasmic GPE, is a substrate of the *ugpQ*-encoded enzyme.

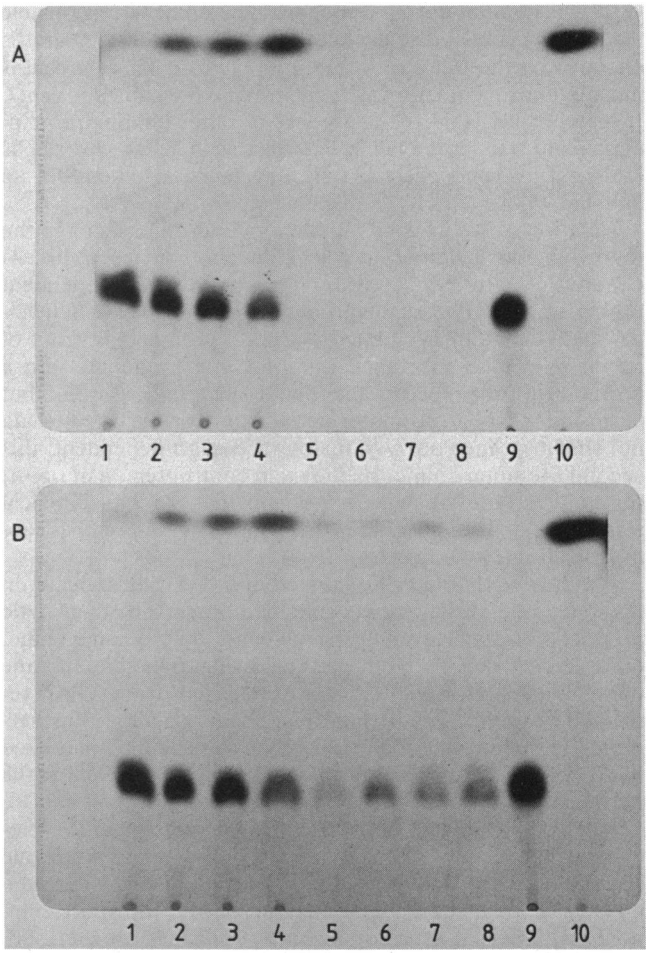

FIG. 7. Hydrolysis of GPE by the wild-type strain and by a strain exhibiting reduced UgpQ expression. Strain Brz49 (A), expressing a wild-type level of UgpQ, and strain Brz426, carrying plasmid pBrz3 (B) and expressing a reduced level of UgpQ but a normal level of Ugp transport proteins, were suspended to an optical density at 578 nm of 35. Ten microliters was incubated with 10 μl of ethanolamine-labeled [^{14}C]GPE for 0.5, 5, 10, and 20 min. Supernatants were analyzed by TLC (condition A). The cell pellets were washed once with 100 μl of MMA and incubated further in MMA for 10 min. Cytoplasmic extracts were then made by adding TCA to a final concentration of 5%. After centrifugation, they were analyzed by TLC (condition A). Lanes: 1 to 4, medium supernatant of the cells; 5 to 8, cytoplasmic extracts; 9, ethanolamine-labeled GPE; 10, ethanolamine. The slow appearance of ethanolamine in the cytoplasm after washing of cells (panel B, lanes 5 to 8) is due to the *ugpQ*-independent activity observed in all strains as discussed above.

DISCUSSION

In this work, we have shown that glycerophosphoryl diesters are substrates of the *ugp*-dependent transport system previously recognized as a G3P transport system under the control of the *pho* regulon. The substrate recognition site for both substrates is established by the periplasmic binding protein, UgpB. G3P is transported in an unmodified form to the cytoplasm, where it supplies the cell with phosphate. Glycerophosphoryl diesters, however, were hydrolyzed to

G3P plus alcohol during transport. The enzyme responsible for this hydrolysis has a molecular weight of 17,500 and is encoded by the last gene of the *ugp* operon. Therefore, it is most likely a member of the *ugp* system. We named it *ugpQ*. It is interesting that the last genes of other binding protein-dependent transport system operons, such as the *rbs* (ribose [16]) and *pst* (phosphate [34, 42]) systems, also code for an enzyme whose activity is dispensable for transport.

Isolation of *lacZ* fusions in the *ugp* operon leads us to the following conclusions. The four known *ugp* genes, *ugpB*, *A*, *C*, and *Q*, are regulated by one main *phoB*-dependent promoter with *ugpB* as the promoter-proximal gene. Schweizer and Boos (38) postulated a second promoter in front of *ugpA*. We have subcloned different *ugp* fragments into a promoter probe vector and could also find a weak but *phoB*-independent promoter activity in front of *ugpA* (data not shown). Since our *ugp* fusions are *phoB* dependent, this second promoter cannot be the main control region of *ugpA*, *C*, and *Q*. It is weak and probably has no physiological significance. We postulate that the four *ugp* genes are organized into one operon.

We showed that UgpQ-mediated hydrolysis must occur on the inner side of the cytoplasmic membrane. The hydrolytic products of GPG and GPE, glycerol and ethanolamine, diffused very quickly out of the cell owing to their membrane permeability. However, choline released from GPC remained inside the cell owing to its positive charge. Furthermore, we could show that, in contrast to GlpQ-mediated hydrolysis of the same substrates, G3P is not released in the periplasm.

We were unable to characterize this protein on the biochemical level since cellular extracts did not exhibit enzyme activity. We presented evidence that this result is due to a physical dependency of the enzyme on the transport components. Internal glycerophosphoryl diesters are not substrates of the *ugpQ*-encoded enzyme; they are hydrolyzed only when transported by the Ugp system.

This situation is somewhat reminiscent of group translocation, the vectorial phosphorylation of sugars by the phosphoenolpyruvate-dependent phosphotransferase systems (PTS) during their translocation through the membrane (32). However, phosphorylation of PTS sugars is required for their translocation, while transport of glycerophosphoryl diesters by the Ugp system does not require their hydrolysis. Nevertheless, as is the case for the PTS components, the enzymatic action of GlpQ is restricted to the incoming substrate.

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