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Escherichia coli Cytosolic Glycerophosphodiester Phosphodiesterase (UgpQ) Requires Mg^{2+}, Co^{2+}, or Mn^{2+} for Its Enzyme Activity▽

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Escherichia coli **cytosolic glycerophosphodiester phosphodiesterase, UgpQ, functions in the absence of other proteins encoded by the *ugp* operon and requires Mg^{2+}, Mn^{2+}, or Co^{2+}, in contrast to Ca^{2+}-dependent periplasmic glycerophosphodiester phosphodiesterase, GlpQ. UgpQ has broad substrate specificity toward various glycerophosphodiesters, producing *sn*-glycerol-3-phosphate and the corresponding alcohols. UgpQ accumulates under conditions of phosphate starvation, suggesting that it allows the utilization of glycerophosphodiesters as a source of phosphate. These results clarify how *E. coli* utilizes glycerophosphodiesters using two homologous enzymes, UgpQ and GlpQ.**

Glycerophosphodiesters are enzymatically produced by phospholipases A_1 and A_2 from membrane phospholipids (10, 25). There are several glycerophosphodiesters, based on the alcohol moiety: glycerophosphocholine (GPC), glycerophosphoethanolamine (GPE), glycerophosphoinositol (GPI), glycerophosphoserine (GPS), glycerophosphoglycerol (GPG), and so on. Glycerophosphodiesters are further degraded by glycerophosphodiester phosphodiesterase (EC 3.1.4.46), producing the corresponding alcohols and *sn*-glycerol-3-phosphate (G3P), which is an essential precursor for de novo synthesis of glycerophospholipids. In the metabolic pathway of *Escherichia coli*, glycerophosphodiesters are thought to be utilized by two distinct systems. One is the Glp system, and the other is the Ugp system (26). In each system, related proteins are encoded by one or more operons. They contain genes that code for transporter proteins and enzymes.

The *glpTQ* operon simultaneously regulates the transcription of related genes (13). GlpT, an ABC transporter, actively transports G3P from the periplasm into the cytosol through the plasma membrane. *glpQ* codes for a periplasmic glycerophosphodiester phosphodiesterase. GlpQ processes periplasmic glycerophosphodiesters into G3P and corresponding alcohols (14).

The other glycerophosphodiester-utilizing system is the Ugp system. The *ugp* operon is constituted of *ugpB*, *ugpA*, *ugpE*, *ugpC*, and *ugpQ* (4, 19). UgpB specifically binds to glycerophosphodiesters and delivers them to the membrane transporter. UgpA, UgpE, and UgpC constitute the transporter for glycerophosphodiesters. UgpQ, a 27-kDa protein, is a cytosolic glycerophosphodiester phosphodiesterase and is homologous to GlpQ.

GlpQ enzymology has been studied in some detail. GlpQ requires Ca^{2+} for its glycerophosphodiester phosphodiesterase activity (14). GlpQ hydrolyzes GPC, GPE, GPG, GPI, and GPS. The putative catalytic regions of UgpQ are very similar to those of GlpQ (26). In addition to the sequence similarity between UgpQ and GlpQ, it has been strongly suggested that UgpQ functions as a glycerophosphodiester phosphodiesterase, based on studies using an *E. coli* mutant strain lacking both *ugpQ* and *glpQ* that is not capable of hydrolyzing glycerophosphodiesters (4). However, the enzyme activity of UgpQ has not been observed in crude extracts of *E. coli* expressing UgpQ. Because of these observations, it has been postulated that UgpQ has enzyme activity only during the transportation of glycerophosphodiesters, when UgpQ would bind to the membrane transporter proteins encoded by *ugpA*, *ugpE*, and *ugpC* genes (4).

In this study, we reveal that purified UgpQ alone has glycerophosphodiester phosphodiesterase activity, contrary to the previous reports. Our findings provide an understanding of how *E. coli* utilizes glycerophosphodiesters.

Recently, the first mammalian glycerophosphodiester phosphodiesterase (GDE1) was reported to constitute a GDE family (29). The members are homologous to bacterial glycerophosphodiester phosphodiesterases, such as UgpQ and GlpQ. Human GDE1 can process GPI and might be involved in a G-protein signaling pathway (29). GDE3 is reported to be involved in the differentiation of osteoblasts (18). GDE2 from chicken has been shown to induce differentiation of motor neurons (21). Our findings also provide insight into the enzymatic study of these mammalian GDEs.

MATERIALS AND METHODS

Materials. Amberlite IRA-96, G3P dehydrogenase, glycerophosphocholine, phosphatidylserine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, glycerophosphocholine ($CdCl_2$ adduct form) and β-NAD were products of Sigma (St. Louis, MO). Amberlite IRC-50 was purchased from Fluka (St. Louis, MO). *E. coli* strain K-12 (ME9012) was supplied by the National Bio-Resource Project (NIG, Japan): E. coli. UgpQ-deficient *E. coli* strain K-12 (JW3414) is part of the KEIO collection (2). PCR primers were purchased from Operon Biotechnologies (Tokyo, Japan). Anti-UgpQ rabbit polyclonal antibody was made by Hokudo Co., Ltd. (Hokkaido, Japan), using purified UgpQ as an antigen. MES (morpholineethanesulfonic acid), PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid)], MOPS (morpholinepropanesulfonic acid), HEPES, TAPS [*N*-tris(hydroxymethyl)methyl-3-amino-propanesulfonic acid], and CHES [2-(cyclohexylamino)ethanesulfonic acid] were purchased from Dojindo (Kumamoto,

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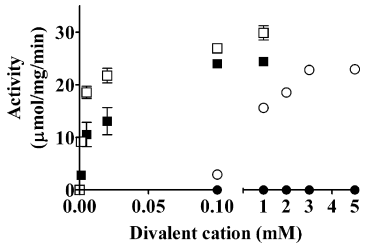

FIG. 1. Effects of divalent cations on UgpQ activity. The glycerophosphodiester phosphodiesterase activity was measured in the presence of 1 mM GPC and various concentrations of divalent cations. The divalent cations shown are $MnCl_2$ (□), $CoCl_2$ (■), $MgCl_2$ (○), and $CaCl_2$ (●). The data are the means of triplicate measurements. The error bars represent standard deviations.

Japan). All other reagents were purchased from Wako Pure Chemicals (Osaka, Japan).

Expression of recombinant UgpQ in *E. coli*. The UgpQ gene was amplified by PCR using the primers 5′-CCGCATATGAGTAACTGGCCTTAT-3′ and 5′-CCCAAGCTTCTATTGGGCCGTAAA-3′ and *E. coli* K-12 (strain ME9012) genomic DNA as a template DNA. The PCR products were subcloned into the NdeI and HindIII sites of pET-11a. The DNA sequence of the clone was determined using an ABI Prism 4700 DNA analyzer (Applied Biosystems, Foster City, CA). *E. coli* BL21(DE3) was transformed by the plasmid. The cells were grown for 40 h at 25°C in 3 liters of LB medium containing 50 μg/ml ampicillin. The cells (13.8 g) were disrupted by sonication in 14 ml of 20 mM Tris-HCl, pH 8.0, 500 mM NaCl, 5 mM 2-mercaptoethanol. The cell debris was removed by centrifugation at 15,000 rpm for 30 min. The supernatant was further centrifuged (100,000 × *g*; 60 min; 4°C) for column chromatography.

Purification of recombinant UgpQ. The resulting supernatant was desalted using a HiPrep 26/10 desalting column (GE Healthcare, Piscataway, NJ) with 20 mM Tris-HCl, pH 8.0 (buffer A). The eluate was subjected to a HiPrep 16/10 Q XL (GE Healthcare) column equilibrated with buffer A. The UgpQ-containing fractions were desalted and then subjected to a Resource Q column (GE Healthcare) equilibrated with buffer A. The UgpQ-containing fractions were desalted with 10 mM sodium phosphate, pH 7.0 (buffer B), and then applied to a CHT20-I (20 ml; Bio-Rad, Hercules, CA) column equilibrated with buffer B. The protein was eluted with a linear gradient of 0.01 to 0.15 M sodium phosphate, pH 7.0. The UgpQ-containing fractions were then applied to a HiLoad 16/60 Superdex 200 (GE Healthcare) column equilibrated with buffer A containing 200 mM NaCl. The protein sample was dialyzed against 50 mM Tris-HCl, pH 8.0, 200 mM NaCl, 1 mM EDTA and then dialyzed against the same buffer without EDTA to remove the EDTA completely.

Determination of the N-terminal amino acid sequence. The purified protein was transferred to a polyvinylidene difluoride membrane using ProSorb (Applied Biosystems). The N-terminal amino acid sequence was analyzed with a Procise 492HT protein sequencer (Applied Biosystems).

Preparation of substrates. GPC (200 mM; $CdCl_2$ adduct form) was dissolved in 200 mM $NaCO_3$ to precipitate $CdCO_3$ (14). To remove Cd^{2+} completely, the supernatant was added to a mixed bed of IRA-96 (anion-exchange resin) and IRC-50 (cation-exchange resin). Cd^{2+}-free GPC was obtained by centrifugation. GPE, GPG, GPS, and GPI were prepared from their phospholipid precursors by a mild alkaline hydrolysis method described previously (11). The concentrations of obtained glycerophosphodiesters were determined enzymatically using UgpQ and G3P dehydrogenase as described in "Enzyme assay" below.

Enzyme assay. Enzyme activity was examined by an enzyme-coupled spectrophotometric assay measuring the amount of G3P generated by the glycerophosphodiester phosphodiesterase reaction. The reaction mixture contained the following: 50 mM HEPES-NaOH, pH 7.4; various concentrations of divalent metal cations; glycerophosphodiesters; and 2 μg/ml UgpQ. The reaction mixture was incubated at 35°C. The reaction was stopped by adding EDTA at a final concentration of 10 mM. The amount of G3P produced by the UgpQ reaction was separately measured using G3P dehydrogenase (14). The assay mixture contained 0.2 M hydrazine-glycine buffer, pH 9.0, 1 mM NAD, 10 U/ml G3P dehydrogenase, and the UgpQ reaction mixture at a final volume of 200 μl. The assay mixture was incubated at 35°C in a 96-well plate for 1 h until oxidation of G3P by G3P dehydrogenase was complete. The G3P concentration was calculated from the absorbance change at 340 nm. The kinetic data were analyzed using Enzyme Kinetics Pro software (ChemSW, Fairfield, CA).

Bacterial strain and growth conditions. *E. coli* K-12 strains, ME9012 (wild type) and JW3414 (UgpQ deficient), were cultured under vigorous agitation at 37°C in a synthetic medium. Starvation for phosphate was provoked by cultivation in a medium containing 0.16 mM KH_2PO_4, 50 mM Tris-HCl, pH 7.7, 8 mM $MgSO_4$, 15 mM $(NH4)_2SO_4$, 27 mM KCl, 7 mM sodium citrate, 2 mM $CaCl_2$, 1 μM $FeCl_2$, 10 μM $MnCl_2$, 4.5 mM glutamic acid, 780 μM tryptophan, 860 μM lysine, and 0.2% glucose (1). The phosphate-rich medium contained the same reagents, except for 0.6 mM KH_2PO_4. *E. coli* was cultivated until its growth in phosphate-poor medium had decreased compared to that under phosphate-rich conditions. The cells were collected by centrifugation at 8,000 rpm for 5 min and were washed with buffer containing 20 mM Tris-HCl, pH 8.0, and 200 mM NaCl (buffer C). The cells were then suspended in buffer C containing 1 mM dithiothreitol. The cells were disrupted by sonication and centrifuged at 10,000 × *g* for 15 min to remove cell debris. The supernatant was centrifuged at 100,000 × *g* for 60 min. The resulting supernatant was regarded as the soluble fraction, and the precipitate was regarded as the membrane fraction. The soluble fraction was desalted with buffer C using PD-10 columns (GE Healthcare).

SDS-PAGE and immunoblotting. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed by the method described by Laemmli (12). Soluble fractions of *E. coli* cultivated under both phosphate-rich and starved conditions were applied to 12.5% SDS-PAGE gels. After electrophoresis, the proteins were transferred to polyvinylidene difluoride membranes (Clear Blot Membrane-P; ATTO, Tokyo, Japan). The membranes were then blocked with 5% nonfat milk (Snow Brand Milk Products, Tokyo, Japan) in Tris-buffered saline (pH 8.0) containing 0.05% Tween 20 at 4°C overnight. A rabbit serum against *E. coli* UgpQ was used as a first antibody at a dilution of 1:1,000 in Tris-buffered saline (pH 8.0) containing 0.05% Tween 20 and 5% nonfat milk. Then, horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G (Sigma; 1:5,000 dilution) was used as a second antibody. The protein bands were visualized using ECL Plus Western blotting detection reagents (GE Healthcare) and a LAS-3000 imaging system (Fujifilm, Tokyo, Japan).

RESULTS

Purification of recombinant UgpQ. Recombinant UgpQ was purified to homogeneity as determined by SDS-PAGE. The single protein band was observed at 27 kDa, which corresponds to the calculated molecular mass of UgpQ (see Fig. 4a). The yield of the purified protein was 30 mg protein/3-liter culture. The purified protein was analyzed by automated Edman degradation. The N-terminal sequence obtained was SNWPYPR, representing the amino terminus of UgpQ without an initiator methionine.

Requirement for divalent cations for the enzyme activity of UgpQ. Enzyme activity of purified UgpQ toward GPC was detected in a time- and enzyme concentration-dependent manner in the presence of 5 mM $MgCl_2$. No enzyme activity was detected in the absence of $MgCl_2$. Thus, we confirmed that UgpQ is glycerophosphodiester phosphodiesterase and that it requires divalent cations, such as Mg^{2+}, for its activity.

The effects of divalent cations on UgpQ activity were studied in detail (Fig. 1). Mn^{2+} and Co^{2+} were most effective for the activity: 100 μM Mn^{2+} or Co^{2+} was sufficient for maximum activity. In the case of Mg^{2+}, however, a higher concentration

TABLE 1. Substrate specificity of UgpQ[a]

Substrate	K_m (mM)	k_{cat} (s^{-1})	k_{cat}/K_m (10^4 M^{-1} s^{-1})
GPC	2.0	3.2	1.6
GPE	0.22	1.1	4.8
GPS	0.66	2.8	4.2
GPG	0.62	3.3	5.4
GPI	0.39	1.8	4.7

[a] Enzyme activity was examined in the presence of 5 mM $MgCl_2$ at 35°C.

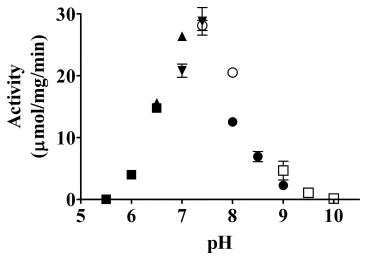

FIG. 2. Effect of pH on UgpQ activity. The reaction mixture contained 50 mM buffer at a pH range from 5.5 to 10, 1 mM GPC, 5 mM $MgCl_2$, and 2 μg/ml UgpQ. The reaction rate was maximal at pH 7.5. The pH dependency of the enzyme reaction showed a typical bell-shaped profile. The buffers used for the experiments were MES-NaOH (■), PIPES-NaOH (▲), MOPS-NaOH (▼), HEPES-NaOH (○), TAPS-NaOH (●), and CHES-NaOH (□). The data are the means of triplicate experiments. The error bars represent standard deviations.

(3 mM) was required for maximum activity. The divalent cation used physiologically was assumed to be Mg^{2+}, considering the intracellular Mg^{2+} concentration (>0.5 mM). Ca^{2+}, which is most effective for GlpQ, did not work (14). Cu^{2+}, Ni^{2+}, Cd^{2+}, Zn^{2+}, and Fe^{2+} (100 μM) also did not exert any enzyme activity.

Substrate specificity and pH dependency. Several kinds of glycerophosphodiesters derived from glycerophospholipids were tested for substrates. The K_m and k_{cat} values for the series of substrates tested are shown in Table 1. UgpQ showed comparable K_m values for these glycerophosphodiesters. These results indicate that UgpQ has broad substrate specificity toward glycerophosphodiesters.

Enzyme activity was measured using GPC as a substrate at a pH range from 5.5 to 10. A typical symmetrical bell-shaped pH dependency was observed (Fig. 2). The maximum enzyme activity was observed at pH 7.5. Below pH 5.5 or above pH 9.5, the enzyme activity was negligible.

Induction and localization of UgpQ. Wild-type and UgpQ-deficient *E. coli* K-12 strains were cultured in both phosphate-rich and phosphate-poor media. The growth curve indicated that after 9 h of culture, growth in phosphate-poor medium was significantly decreased compared to that in phosphate-rich medium (Fig. 3a). After 9 h of culture, the cells were collected and disrupted by sonication. Significantly higher glycerophosphodiester phosphodiesterase activity was detected in the soluble fraction of the phosphate-starved wild-type *E. coli* than in that of UgpQ-deficient *E. coli* grown in the phosphate-rich medium (Fig. 3b). The activity was dependent on Mg^{2+} and Mn^{2+}, not on Ca^{2+}, indicating the same divalent cation preference as UgpQ, described above (Fig. 1). On the other hand, GlpQ was not active in the presence of Mg^{2+} but was active in the presence of Ca^{2+} (14). The Mg^{2+}-dependent activity was not observed in the soluble fraction of UgpQ-deficient *E. coli*. Furthermore, expression of the UgpQ protein was significantly induced in the soluble fraction of phosphate-starved wild-type *E. coli*, not in that of UgpQ-deficient *E. coli* (Fig. 4b). Thus, it was clearly shown that the glycerophosphodiester phosphodiesterase activity that appeared in the phosphate-starved wild-type *E. coli* was derived from UgpQ.

DISCUSSION

Glycerophosphodiesters are transported from the periplasmic space to the cytosol by transport proteins composed of UgpA, UgpE, and UgpC and are processed by UgpQ into G3P and the corresponding alcohols (5). The preference of the transporter is reported to be as follows; GPE > GPC > GPG (5). On the other hand, the substrate specificities of UgpQ were comparable among the glycerophosphodiesters tested

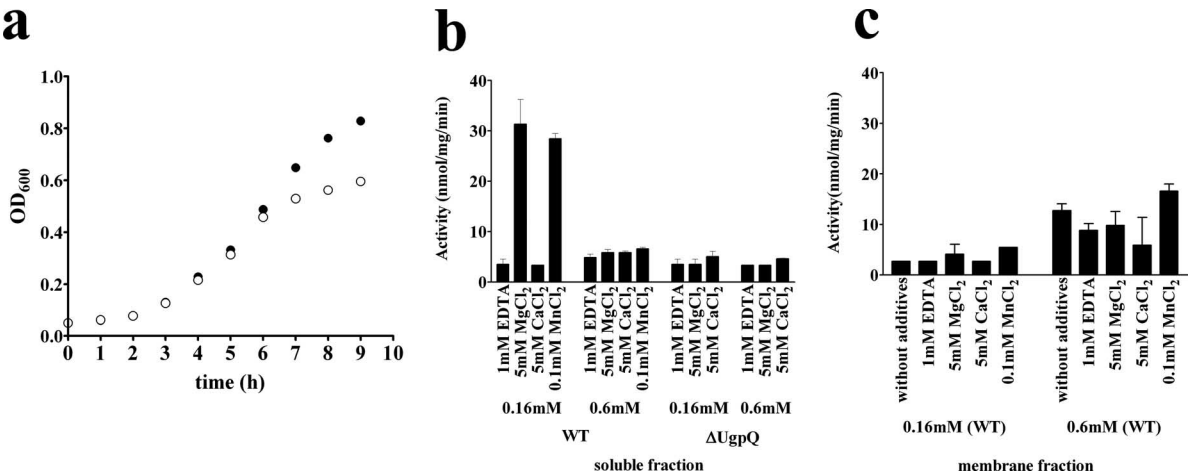

FIG. 3. Induction of glycerophosphodiester phosphodiesterase activity in phosphate-starved *E. coli*. (a) Growth curve of wild-type *E. coli* cultured in phosphate-rich and -poor media. *E. coli* was cultured in a synthetic medium, as described in Materials and Methods. Briefly, starvation for phosphate was provoked by cultivation in a medium containing 0.16 mM P_i (○). The phosphate-rich medium contained 0.6 mM P_i (●). The data are representative of three independent experiments that produced similar results. OD_{600}, optical density at 600 nm. (b and c) Glycerophosphodiester phosphodiesterase activities in the soluble and membrane fractions of wild-type (WT) and UgpQ-deficient *E. coli*. The soluble (b) and membrane (c) fractions of wild-type and UgpQ-deficient *E. coli* cells that were cultured for 9 h were assayed for glycerophosphodiester phosphodiesterase activity at 35°C in the presence of 1 mM EDTA, 5 mM $MgCl_2$, 5 mM $CaCl_2$, or 100 μM $MnCl_2$. The data are the means of triplicate experiments. The error bars represent standard deviations.

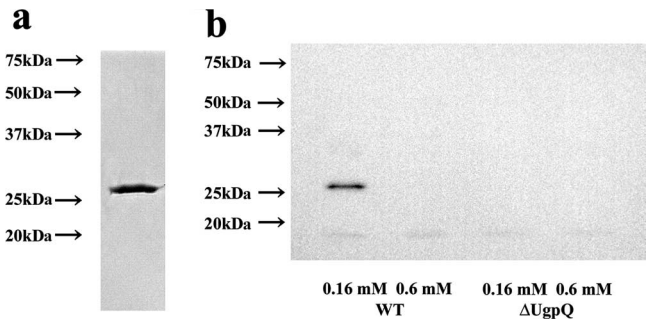

FIG. 4. SDS-PAGE and immunoblot analysis. (a) SDS-PAGE of purified recombinant UgpQ. A single band was observed at 27 kDa by Coomassie brilliant blue staining. (b) Detection of UgpQ in phosphate-starved *E. coli* by immunoblotting. The soluble fractions of wild-type (WT) and UgpQ-deficient *E. coli* under phosphate-starved (0.16 mM) and -rich (0.6 mM) conditions (4 μg of protein in each lane) were subjected to immunoblot analysis using rabbit antiserum against UgpQ (1:2,000 dilution).

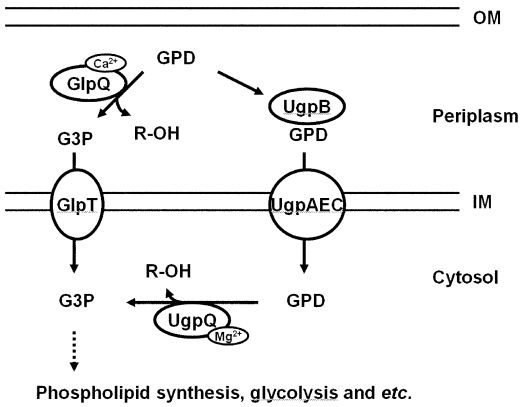

FIG. 5. Proposed metabolic pathway of glycerophosphodiesters by UgpQ and GlpQ in *E. coli*. GPD, glycerophosphodiesters; IM, inner membrane; OM, outer membrane; R-OH, ethanolamine, glycerol, choline, serine, inositol, etc.

(Table 1). Thus, the overall specificity of the Ugp system is assumed to be explained by the specificity of the transporter protein. One source of substrates for the Ugp system could be glycerophospholipids that are deacylated extracellularly by phospholipases A_1 and A_2 to form glycerophosphodiesters (10, 25). A phospholipase (OMPLA) that has phospholipase A_1, A_2, and lysophospholipase activity exits through the outer membrane in gram-negative bacteria, including *E. coli* (8). Lysophospholipids, substrates for OMPLA, are produced during biosynthesis of lipopolysaccharide and lipoprotein by phospholipid::lipid A palmitoyltransferase (PagP) and apolipoprotein *N*-acyltransferase (Lnt), respectively (3, 27). Teichoic acid that contains GPG is also abundantly present in the cell walls of gram-positive bacteria (24).

Extracellular glycerophosphodiesters can enter into two pathways (Fig. 5). One is carried out by the Glp system, and the other is carried out by the Ugp system. In the Ugp system, glycerophosphodiesters are thought to be transported by using energy from hydrolysis of ATP by UgpC (6). On the other hand, when G3P produced by GlpQ from glycerophosphodiesters in the periplasm is transported by GlpT, the net phosphate uptake is zero, because GlpT is a P_i antiporter (9). Thus, the Ugp system would be superior for an efficient uptake of phosphate. Since the *ugp* operon has a *phoB*-dependent promoter (23) and UgpQ is up-regulated under phosphate-starved conditions (Fig. 3 and 4), the primary physiological function of UgpQ would be utilization of glycerophosphodiesters as a source of phosphate. G3P produced by UgpQ would be used for the de novo synthesis of glycerophospholipids through the acylation by G3P acyltransferases (28). G3P is also metabolized by G3P dehydrogenases, producing dihydroxyacetone-phosphate, an intermediate of glycolysis (5). Actually, glycerophosphodiesters that were provided in the culture medium of *E. coli* were converted into G3P and then incorporated into phospholipids, protein (23), and also all phosphate-containing molecules, including nucleotides, because *E. coli* can grow on glycerophosphodiesters as a sole phosphate source (5). Because the glycerophosphodiester phosphodiesterase activity and the UgpQ protein were not observed in *E. coli* solely under phosphate-rich conditions (Fig. 3 and 4), the Ugp system might not be important under those circumstances.

The in vivo enzyme activity of UgpQ was reported using *E. coli* cells in which the *ugp* operon was overexpressed (4). GPE added to the culture medium was incorporated into the cells and was processed into G3P. However, the enzyme activity was not observed in the extracts of the *E. coli* cells. That is the reason why UgpQ was proposed to be functional only during transport into the cytosol by UgpA, UgpE, and UgpC (4). Our data show that the UgpQ protein and its enzyme activity exist in the soluble fraction, not in the membrane fraction (Fig. 3b and c), indicating that UgpQ functionally localizes in the cytosol, because no export signal sequence is present in the N terminus of UgpQ. Thus, it is not necessary to assume coupling of the UgpQ activity and transport by UgpA, UgpE, and UgpC. We suppose that the same assay conditions as for GlpQ, which contains Ca^{2+}, not Mg^{2+}, might have led to a failure to detect the enzyme activity of UgpQ in vitro in the previous studies (4, 14).

Among mammalian GDEs, enzymatic properties have been reported only for GDE1 (29). Our results suggest that various divalent cations, Ca^{2+}, Mg^{2+}, Mn^{2+}, Co^{2+}, etc., should also be considered for the enzymatic analysis of the other mammalian GDEs.

GpdQ is another type of bacterial cytosolic phosphodiesterase from *Enterobacter aerogens* and has broad substrate specificity toward some kinds of organophosphates, such as paraoxon, demeton, dimethyl phosphate (DMP), bis(*p*-nitrophenyl) phosphate, *p*-nitrophenyl phosphate, and GPE (15). GpdQ has been studied in the interest of bioremediation of soil by detoxication of organophosphate pesticides. Although no apparent sequence similarity between UgpQ and GpdQ exists to indicate homology, the structure of the *gpdQ*-containing operon is very similar to that of the *ugp* operon. The *gpdQ*-containing operon has all genes orthologous to the *ugp* operon except for the UgpQ gene. Thus, it is questioned whether UgpQ can process these organophosphates in addition to glycerophosphodiesters. A growth assay of *E. coli* using DMP as a sole phosphate source suggested that transporter proteins of the Ugp system can transport DMP but UgpQ cannot process DMP sufficiently

for growth (15). It has also been reported that bis(*p*-nitrophenyl) phosphate is not a substrate for GlpQ (14). Thus, we assume that UgpQ might be specific for glycerophosphodiesters, unlike GpdQ.

The crystal structures of UgpQ orthologs from *Thermotoga maritima* (22), *Thermus thermophilus* (Protein Data Bank [PDB] accession no. 1VD6), and *Agrobacterium tumefaciens* (20) were recently reported. However, the structures do not indicate binding of essential divalent cations, such as Mg^{2+}. The crystal structures of GlpQ are also available in the PDB database (PDB accession no. 1T8Q and 1YDY). The overall structures of both UgpQ and GlpQ are triosephosphate isomerase barrel folds. Recently, the crystal structure of sphingomyelinase D from spider venom was reported (16, 17). Sphingomyelinase D requires Mg^{2+} for its enzyme activity (7). The overall structure is a triosephosphate isomerase barrel fold and is very similar to UgpQ orthologs and GlpQ. In the crystal structure of sphingomyelinase D, one Mg^{2+} is coordinated by two Asp (D34 and D91), one Glu (E32), and three water molecules and stabilizes the phosphate group of the substrate (16). The structure of the Mg^{2+}-binding site of sphingomyelinase D is very close to that of the Ca^{2+}-binding site of GlpQ. These structures and our kinetic data strongly suggest that UgpQ has one Mg^{2+} coordinated by E39, D41, and E114 to stabilize the phosphate group of substrate glycerophosphodiesters. It seems that two residues with pK_a values around 6.5 and 8 participate in the catalysis (Fig. 2). Considering the neutral pK_a values, we suggest that they are histidine residues (H12 and H54), as proposed for the catalytic residues of sphingomyelinase D (16). However, the Mg^{2+}-binding form and the substrate recognition of UgpQ remain to be elucidated.

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