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Glycogen Phosphorylase, the Product of the *glgP* Gene, Catalyzes Glycogen Breakdown by Removing Glucose Units from the Nonreducing Ends in *Escherichia coli*†

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To understand the biological function of bacterial glycogen phosphorylase (GlgP), we have produced and characterized *Escherichia coli* cells with null or altered *glgP* expression. *glgP* deletion mutants (Δ*glgP*) totally lacked glycogen phosphorylase activity, indicating that all the enzymatic activity is dependent upon the *glgP* product. Moderate increases of glycogen phosphorylase activity were accompanied by marked reductions of the intracellular glycogen levels in cells cultured in the presence of glucose. In turn, both glycogen content and rates of glycogen accumulation in Δ*glgP* cells were severalfold higher than those of wild-type cells. These defects correlated with the presence of longer external chains in the polysaccharide accumulated by Δ*glgP* cells. The overall results thus show that GlgP catalyzes glycogen breakdown and affects glycogen structure by removing glucose units from the polysaccharide outer chains in *E. coli*.

Glycogen is a branched homopolysaccharide of α-1,4-linked glucose subunits with α-1,6-linked glucose at the branching points. Synthesized by glycogen synthase (GlgA; EC 2.4.1.21) using ADP-glucose (ADPG) as the sugar donor molecule, the accumulation of this polyglucan occurs under conditions of limited growth in the presence of an excess carbon source (44).

Regulation of glycogen biosynthesis in *Escherichia coli* involves a complex assemblage of factors that are adjusted to the physiological status of the cell. At the level of enzyme activity, for instance, the glycogen biosynthetic process is subjected to the regulation of ADPG synthesizing and cleaving enzymes, i.e., ADPG pyrophosphorylase (GlgC; EC 2.7.7.27) (5) and ADP-sugar pyrophosphatase (AspP; EC 3.6.1.21) (41), respectively. Moreover, depending on the carbon source, phosphoglucomutase (PGM; EC 2.7.5.1) has been shown to limit the glycogen biosynthetic process (1, 13). At the level of gene expression, the gluconeogenic process depends on the regulation of *glgBX* (coding, respectively, for the glycogen branching and debranching enzymes), *glgS* and *glgCAP* operons (34, 37, 44), the latter being regulated by cyclic AMP, GDP 3′-diphosphate, and the carbon storage regulator CsrA (3, 47, 54).

glgCAP encodes the two anabolic enzymes required for glycogen synthesis, GlgC and GlgA. Surprisingly at first glance, however, this operon also encodes the presumed catabolic enzyme glycogen phosphorylase (GlgP; EC 2.4.1.1) (46). This enzyme belongs to a structurally related and ubiquitous group of proteins that are generally regarded as glucan-degrading enzymes that catalyze the production of glucose-1-phosphate (G1P) by the reversible cleavage of α-1,4 bonds at the nonreducing ends of polyglucans, such as maltodextrins, starch, and glycogen (14, 20, 36).

The highly regulated muscle and liver glycogen phosphorylases are by far the best understood and most extensively studied members of the glucan phosphorylases group, their involvement in mammalian glycogen breakdown being well documented (6, 9, 10, 16, 40, 42). By contrast, despite the fact that plant glucan phosphorylases are assumed to be involved in generating G1P, some of them have been shown to play a pivotal role in processes such as tolerance to abiotic stress, flowering, and seed growth (28, 50, 52, 56). Despite intensive efforts, a case for a function of plant starch phosphorylases remains to be made.

In contrast to the case of mammalian glycogen phosphorylases, the role of the bacterial GlgP in glycogen metabolism is not well understood. Palmer et al. (43), for instance, suggested that GlgP does not play an important role in glycogen breakdown in *E. coli*. In clear contrast, and based on both its structural similarities with the mammalian counterpart and on the intracellular orthophosphate (P_i)-to-G1P ratio, bacterial GlgP has generally been suggested to recess the outer glycogen chains down to a length of four glucose residues from the branch point and, in combination with a debranching enzyme

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† Supplemental material for this article may be found at http://jb.asm.org/.

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TABLE 1. Bacterial strains and plasmids used in this study

Designation	Relevant genotype or description	Source
Bacteria		
B/r	B derivative, *lon-11 sulA1* λ^r *mal* (Ts)	CGSC
B/r Δ*glgP*	B/r Δ*glgP*::Kan^r, *glgP* in the *glgCAP* operon replaced by a kanamycin resistance cassette	This work
B/r Δ*glgCAP*	B/r Δ*glgCAP*::Kan^r, *glgCAP* operon replaced by a kanamycin resistance cassette	This work
BL21(DE3) C43	F′ *ompT hsdS*$_B$(r_B^- m_B^-) *gal dcm* (DE3); BL21(DE3) derivative	Avidis
BL21(DE3) C43 Δ*glgP*	BL21(DE3) C43 Δ*glgP*::Kan^r	This work
BL21(DE3) C43 Δ*glgCAP*	BL21(DE3) C43 Δ*glgCAP*::Kan^r	This work
TG1	K-12 derivative; F′ *traD36 lacI*^{q} Δ(*lacZ*)*M15 proBA*/*supE* Δ(*hsdM-mcrB*)*5* [r_k^- m_k^- $mcrB^-$] *thi* Δ(*lac-proBA*)	Pasteur Institute
TG1 Δ*glgP*	TG1 Δ*glgP*::Kan^r	This work
BW25113	*lacI*^{q} *rrnB*$_{T14}$ Δ*lacZ*$_{WJ16}$ *hsdR514* Δ*araBAD*$_{AH33}$ Δ*rhaBAD*$_{LD78}$	23
BW25113 Δ*glgP*	BW25113 Δ*glgP*::Kan^r	This work
Plasmids		
pBAD-18	Expression plasmid, Amp^r	33
pBAD-GlgP	PBAD-18 directing *glgP* expression	This work
pET-15b	Expression plasmid, Amp^r	Novagen
pET-GlgP	PET-15b directing *glgP* expression	This work

(GlgX), to participate in the slow degradation of glycogen during extended periods of substrate deprivation (4, 18, 21, 24, 38, 54, 55). The phenotype recently recorded for GlgX-defective *E. coli* mutants (24) is certainly consistent with this view. Nevertheless, the implication of bacterial GlgP in glycogen breakdown has never been formally demonstrated. Indeed, production and biochemical characterization of both *glg*P-less and -overexpressing cells are still lacking, and the role of GlgP in regulating glycogen breakdown must be considered as only imaginable and based on indirect evidence (4, 49). Significantly, Boss and coworkers have provided evidence that GlgP is involved in the production of maltodextrins acting as substrates and inducers for maltodextrin metabolism enzymes (12, 15, 25, 27, 29).

To gain insight into both functional and biological aspects of bacterial GlgP, we have characterized cells with altered glycogen phosphorylase activities. Results presented in this work demonstrate that GlgP catalyzes glycogen breakdown and affects glycogen structure by removing glucose units from the polysaccharide outer chains in *E. coli*.

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture media. Bacterial strains and plasmids used in this work and their characteristics are summarized in Table 1. Plasmids were propagated in *E. coli* XL1-Blue with the appropriate antibiotic selection. DNA manipulations were conducted by following the procedures indicated by Ausubel et al. (2). For biochemical analyses, cells were grown in either liquid Kornberg (1.1% K_2HPO_4, 0.85% KH_2PO_4, 0.6% [wt/vol] yeast extract) medium or M9 (48 mM Na_2HPO_4, 22 mM KH_2PO_4, 7 mM NaCl, 17 mM NH_4Cl, 0.1 mM $CaCl_2$, 2 mM $MgSO_4$) minimal medium supplemented with 50 mM glucose. When indicated, cells were cultured with the indicated amounts of arabinose. LB medium (1% tryptone, 1% NaCl, 0.5% yeast extract) was used for routine laboratory cultures. In every case, cells were grown aerobically with rapid gyratory shaking at 37°C after inoculation with 1 volume of an overnight culture per 50 volumes of fresh medium. Solid medium was prepared by addition of 1.5% bacteriological agar to liquid medium.

***glgP* and *glgCAP* disruptions.** Insertional mutagenesis of the *glgCAP* operon and *glgP* was carried out by using the method developed by Datsenko and Wanner (23). A selectable antibiotic resistance gene was generated by PCR from a freshly isolated colony of *E. coli* MC4100 containing a chromosomally located kanamycin resistance cassette, using 80-nucleotide-long primer pairs that included 60-nucleotide homology extensions for the targeted locus and 20-nucleotide priming sequences for the kanamycin resistance gene (Table 2). *glgCAP* and *glgP* deletion mutants (Δ*glgCAP* and Δ*glgP*, respectively) were confirmed by both Southern hybridization and PCR using *glgCAP*- and *glgP*-specific primers, respectively.

***glgP* expression.** *glgP* was amplified by PCR using the chromosomal DNA of *E. coli* BL21(DE3)C43 as template and the following primers: forward, 5′-GCTA GCAGGAGCTCGAGTCCATGAATGCTCCGTTTACATATTC-3′; reverse, 5′-GGATCCTTACAATCTCACCGGATCGATATGC-3′.

The ca. 2.5-kb PCR product thus obtained was cloned in the pGEM-T Easy vector (Promega) to create pG-glgP. *glgP* was subsequently cloned into either pET-15b (Novagen) or pBAD-18 (a pBAD/His derivative; Invitrogen) expression vectors (33), as illustrated in Fig. S1 and S2 in the supplemental material, to produce pET-glgP and pBAD-glgP, respectively. These plasmids were then used to transform cells by electroporation.

Production and purification of a recombinant GlgP. His-tagged GlgP was purified using BL21(DE3)C43 transformed with pET-glgP. Cells were grown in LB medium containing 0.1 mg/ml of ampicillin to an absorbance at 600 nm of

TABLE 2. Primer pairs for *glgCAP* and *glgP* disruption[a]

Locus	Direction	Sequence
glgCAP	Forward	GTG CAG CAC ATT CAG CGC GGC TGG TCA TTC TTT AAT GAA GAA ATG AAC GAG TTT GTC GAT **AAA GCC ACG TTG TGT CTC AA**
	Reverse	ATT CGC ACC GTC CAA CGT ACC GAT AGT CAG CGC ACC GTT AAG CGC AAA CTT CAT GTT ACT **GCG CTG AGG TCT GCC TCG TG**
glgP	Forward	ACG TTA GGG TTG CCG GGG CGC GGT TAC GGC ATC CGC TAT GAC TAC GGT ATG TTC AAG CAG **AAA GCC ACG TTG TGT CTC AA**
	Reverse	ATT CGC ACC GTC CAA CGT ACC GAT AGT CAG CGC ACC GTT AAG CGC AAA CTT CAT GTT ACT **GCG CTG AGG TCT GCC TCG TG**

[a] Priming sequences for the kanamycin resistance gene are indicated in bold.

about 0.6, and then 0.5 mM isopropyl-β-D-thiogalactopyranoside was added to the culture medium. After 3 hours, the cells accumulated a protein whose size (ca. 90 kDa) was consistent with the predicted molecular mass (92.3 kDa) of the *glgP* gene product (see Fig. S3 in the supplemental material). One hundred milliliters of cultured cells was centrifuged at 6,000 × *g* for 10 min. The pelleted bacteria were resuspended in 4 ml of His-bind binding buffer (Novagen), sonicated, and centrifuged at 10,000 × *g* for 10 min. The supernatant thus obtained was subjected to His-bind chromatography (Novagen). The eluted protein was then rapidly desalted by ultrafiltration on a Centricon YM-10 filter (Amicon, Bedford, MA).

Enzyme assays. The following enzymes were assayed at 37°C according to the indicated references: GlgC (48), AspP (41), GlgA (35), and PGM (1). GlgP was assayed in the direction of glucan breakdown by using a two-step determination of G1P. In step one, the reaction mixture (50 μl) contained 50 mM HEPES (pH 7.5), 30 mM Na-phosphate buffer (pH 7.5), glycogen (equivalent to 10 mM glucose), and protein extract. After 15 min of incubation, the reaction was stopped by boiling for 2 min. The G1P liberated was determined by using either one of the following methods.

(i) Assay A. Spectrophotometrically, assay A was conducted with a 300-μl mixture containing 50 mM HEPES (pH 7.0), 1 mM EDTA, 2 mM $MgCl_2$, 15 mM KCl, 0.6 mM NAD^+, 1 U each of desalted PGM (P3397; Sigma) and glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides* (G8404; Sigma), and 30 μl of the mixture from the step one reaction. After 20 min of incubation, the NADH production was monitored at 340 nm by using a Multiskan EX spectrophotometer (Labsystems, Chicago, Ill.).

(ii) Assay B. Assay B entailed high-performance liquid chromatography with pulsed amperometric detection of G1P on a DX-500 Dionex system fitted to a Carbo-Pac PA10 column (4 by 250 mm) (7).

One unit of enzyme activity was defined as the amount of enzyme that catalyzes the production of 1 μmol of product per min.

Analytical procedures. Bacterial growth was determined spectrophotometrically by following the absorbance at 600 nm. Protein content was measured by the Bradford method using a Bio-Rad prepared reagent. Glycogen was extracted and measured as described by Romeo et al. (48). Iodine staining of colonies on solid Kornberg medium was performed using iodine vapor essentially as described in references 26 and 48.

CLDs of Δ*glgP* and WT glycogens. Glycogens from wild-type (WT) and Δ*glgP* cells were purified as previously described (24). The purified polysaccharides were then subjected to enzymatic debranching by incubation overnight at 42°C in the presence of 20 U of isoamylase (Megazyme International Ireland Ltd.) from *Klebsiella pneumoniae* in 55 mM sodium acetate pH 3.5 buffer. The chain length distribution (CLD) of glycogen β-limit dextrins was obtained with the same procedure except that the purified glycogens were subjected to β-amylolysis prior to debranching. Two milligrams of purified glycogen was incubated with 17 U of β-amylase from sweet potato (Sigma, St. Louis) overnight in 55 mM sodium acetate pH 4.6 buffer. The reaction was stopped by incubation of the samples during 5 min at 100°C. The β-limit dextrins obtained were then dialyzed versus the same buffer but at pH 3.5.

The CLDs were then analyzed by fluorophore-assisted capillary electrophoresis (FACE). Glucans were first derivatized with 8-amino-1,3,6-pyrenetrisulfonic acid (APTS) according to the manufacturer's recommendations (Beckman Coulter, Fullerton, CA). Briefly, 2 μl APTS in 15% acetic acid solution and 2 μl of 1 M $NaBH_3CN$ in tetrahydrofolate were added, and the coupling reaction was allowed overnight at 37°C in the dark. Separation and quantification of APTS-coupled glucans were carried out on a P/ACE system 5000 (Beckman Coulter) equipped with a laser-induced fluorescence system using a 4-mW argon ion laser. The excitation wavelength was 488 nm, and the fluorescence emitted at 520 nm was recorded on the Beckman P/ACE station software system (version 1.0). Uncoated fused silica capillaries of 57 cm length by 75-μm inner diameter were used. Running buffers were from Beckman Coulter. Samples were loaded into the capillaries by electroinjection at 10 kV for 10 s, and a voltage of 30 kV was applied for 30 min at a constant temperature of 25°C.

EM analyses. Electron microscopy (EM) was done as described by Bonafonte et al. (11). Cells entering the stationary phase of growth were prefixed with 4% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3) for 2 h at 4°C, washed in 0.25 M sucrose, 0.1 M cacodylate buffer, and postfixed in 1% OsO_4 in phosphate buffer (pH 7.3) at 4°C. After two washes with sodium Veronal (pH 7.4), the cells were embedded in 2% molten Noble agar. Sections were treated with 1% periodic acid and then with sodium chlorite. The gel was dehydrated and embedded in Epon-812, and ultrathin sections were placed on nickel grids, poststained for 30 min with uranyl acetate and for 15 min with lead citrate, and observed under a Zeiss EM 10CR electron microscope.

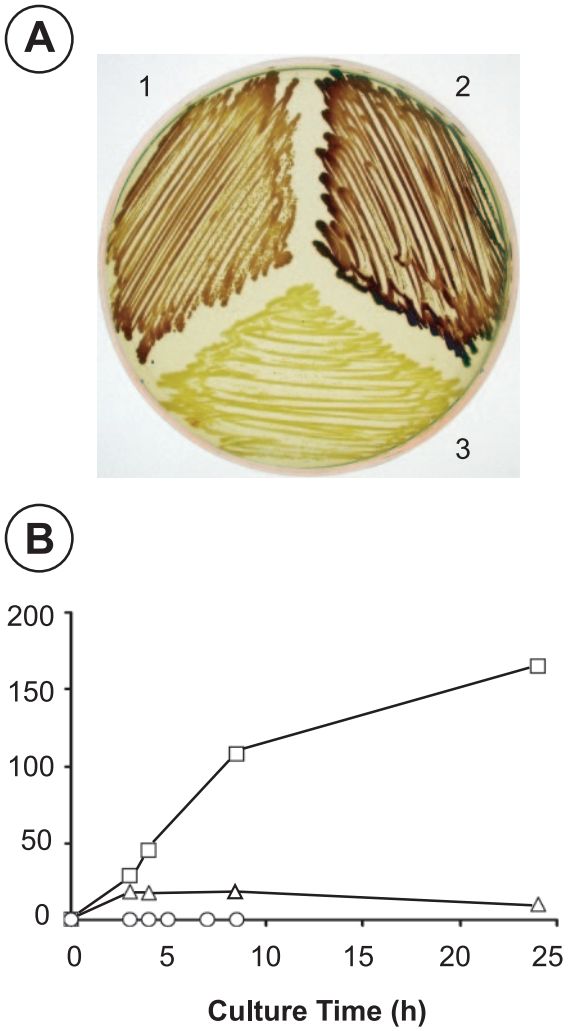

FIG. 1. GlgP catalyzes *E. coli* glycogen breakdown in vivo. (A) Iodine staining of (1) WT, (2) Δ*glgP*, and (3) Δ*glgCAP* cells. Bacteria were cultured overnight in solid Kornberg medium supplemented with 50 mM glucose. (B) Time course analyses of the glycogen content in WT (△), Δ*glgP* (□), and Δ*glgCAP* (○) cells cultured in liquid Kornberg medium supplemented with 50 mM glucose. The bacterial strain employed in this study was B/r. Essentially similar results were obtained with other bacterial strains.

RESULTS AND DISCUSSION

All the glycogen phosphorylase activity in *E. coli* is catalyzed by GlgP. Δ*glgP*- and *glgP*-overexpressing cells were produced as described in Materials and Methods and assayed for glycogen phosphorylase activity in the glycogen breakdown direction. WT cells showed readily measurable enzymatic activities (ca. 10 mU/mg protein). In clear contrast, glycogen phosphorylase activity was undetectable in the Δ*glgP* cells. In addition, *glgP*-overexpressing cells exhibited a ca. 20-fold increase of enzymatic activity compared to WT cells. The overall results thus show that most, if not all, glycogen phosphorylase activity in *E. coli* grown on glucose-containing medium is catalyzed by the *glgP* gene product.

GlgP catalyzes glycogen breakdown in *E. coli*. To elucidate the biological function of GlgP, we compared the glycogen

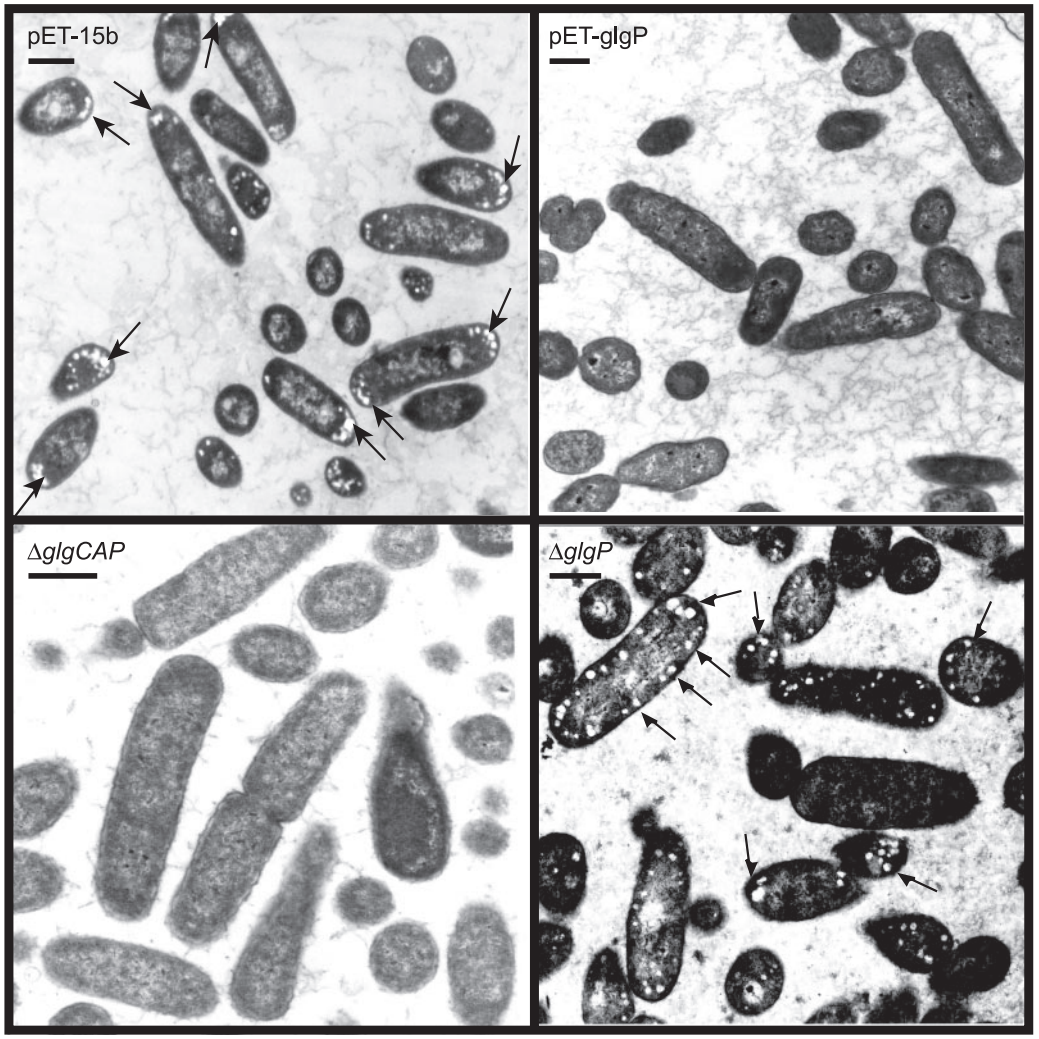

FIG. 2. Glycogen accumulation in GlgP-overproducing cells and Δ*glgP* and Δ*glgCAP E. coli* mutants. Cells cultured in liquid Kornberg medium supplemented with 50 mM glucose were analyzed by EM. Upper left quadrant, WT cells transformed with the plasmid vector (pET-15b); upper right quadrant, GlgP-overproducing WT cells (pET-glgP); lower left quadrant, Δ*glgCAP* mutants; lower right quadrant, Δ*glgP* mutants. Arrows indicate the position of electron-transparent cytoplasmic glycogen granules resembling "holes" preferentially located at the periphery of *E. coli* cells (17). The bacterial strain employed in these studies was BL21(DE3)C43. Essentially similar results were obtained with other bacterial strains. Bars, 1 μm.

contents between WT cells and cells with altered *glgP* expression cultured in Kornberg medium supplemented with 50 mM glucose. Bacteria with altered *glgP* expression displayed a normal growth behavior compared with WT cells (not shown).

Iodine stain analyses on solid cultures (Fig. 1A) revealed that, whereas Δ*glgCAP* cells lacking the whole glycogen biosynthetic machinery (section 3) gave a negative stain compared with WT cells (section 1), Δ*glgP* cells (section 2) displayed a dark, brown iodine stain phenotype (22, 31, 39). We then performed time course measurements of the glycogen content in cells with altered glycogen phosphorylase activity cultured in liquid Kornberg medium supplemented with 50 mM glucose (Fig. 1B). These analyses showed that, as confirmed by EM analyses (Fig. 2), Δ*glgCAP* cells displayed a glycogen-less phenotype. In addition, and consistent with the results presented in Fig. 1, both glycogen amount and the rate of glycogen accumulation in Δ*glgP* cells were severalfold higher than those of their corresponding WT cells. The overall data thus show that (i) GlgP catalyzes glycogen breakdown in *E. coli* and (ii) in agreement with previous reports (8, 30, 32), bacterial cells can synthesize and mobilize glycogen simultaneously.

glgP-overexpressing cells displayed a glycogen-less phenotype similar to that of Δ*glgCAP* cells at any stage of the culture (Fig. 3A), as also indicated by EM analyses (Fig. 2). Whether small increases in glycogen phosphorylase activity are accompanied by a reduction of glycogen levels was investigated by measuring glycogen contents in cells transformed with pBAD-glgP. These cells were cultured in liquid medium supplemented with 50 mM glucose, and glycogen phosphorylase activity was tuned by varying the concentration of arabinose in the culture medium. As shown in Fig. S4 in the supplemental material, both enzymatic activity and glycogen content in cells transformed with pBAD-18 remained nearly constant at any arabinose concentration. In clear contrast, glycogen phosphorylase activity in cells transformed with pBAD-glgP correlated well with arabinose concentration. Most importantly, intracel-

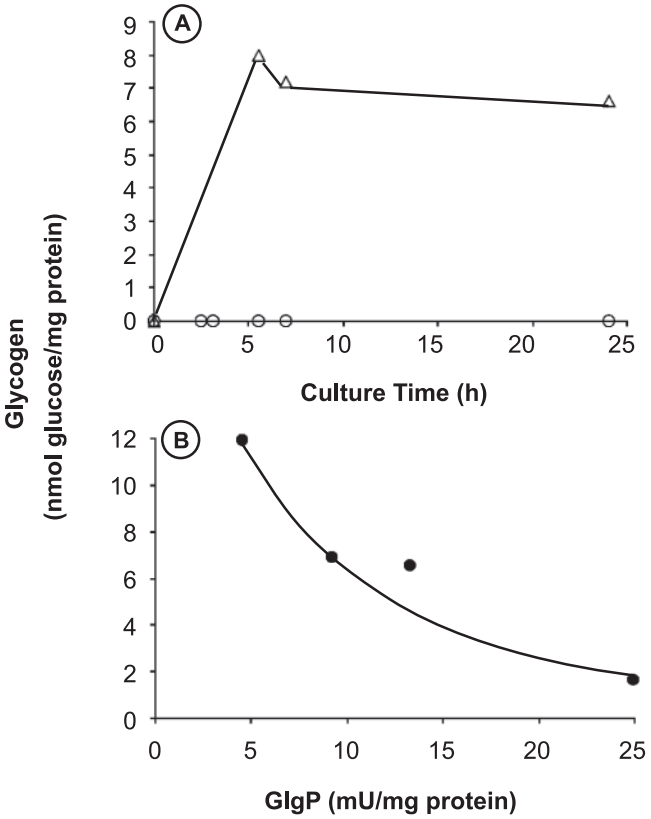

FIG. 3. Increasing glycogen phosphorylase activity leads to a marked reduction of glycogen levels in *E. coli*. (A) Time course analyses of the glycogen content in cells transformed with either pET-15b (△) or pET-glgP (○) cultured in liquid M9 minimal medium supplemented with 50 mM glucose. (B) Glycogen phosphorylase activity-dependent glycogen content in cells transformed with pBAD-glgP cultured in liquid M9 minimal medium supplemented with 50 mM glucose. *glgP* expression was regulated by adding the arabinose concentrations indicated in Fig. S4 in the supplemental material. At the end of the exponential growth phase, cells were harvested and both glycogen content and glycogen phosphorylase activities were measured. The bacterial strain employed in this study was BL21(DE3)C43. Essentially similar results were obtained with other bacterial strains.

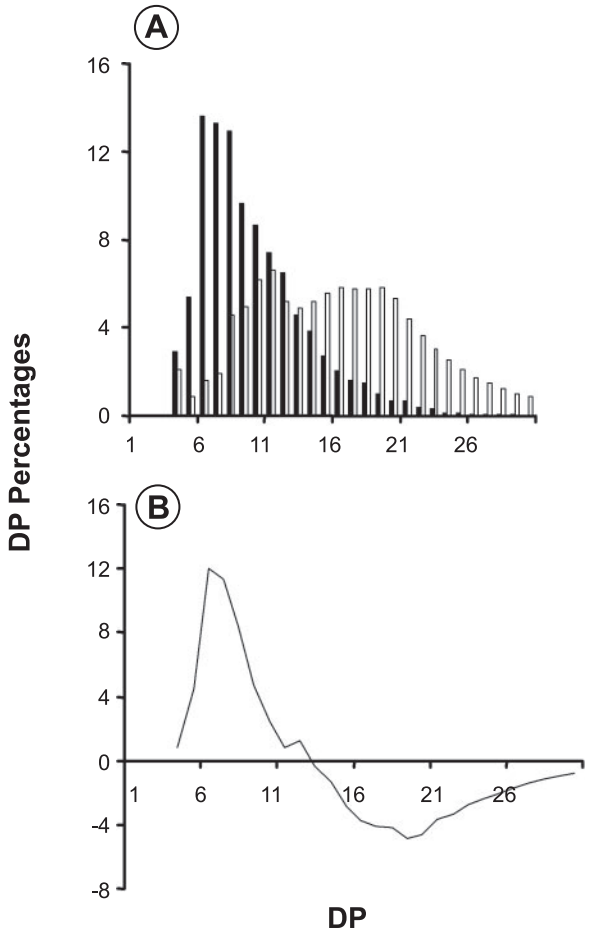

FIG. 4. Chain length distribution of glycogens, determined by FACE (see Materials and Methods). Isoamylase debranched glycogens from both WT and Δ*glgP* cells were produced as described in Materials and Methods and separated up to DP 30. (A) CLDs expressed on a DP scale (*x* axis) are displayed as the percentage of total chains, expressed on a molar basis (*y* scale). (B) Difference plot generated by subtracting the mol% value of the WT at each chain length from the corresponding mol% of the mutant at the corresponding chain length. Dark bars correspond to WT cells, and light bars correspond to Δ*glgP* cells. The bacterial strain used in this study was TG1. Essentially similar results were obtained with other bacterial strains.

lular glycogen content in cells transformed with pBAD-glgP showed a reverse correlation with respect to glycogen phosphorylase activity, and small increases of enzymatic activity were accompanied by a marked reduction of the glycogen content (Fig. 3B). The overall data thus show that GlgP exerts a very strong influence on the glycogen breakdown process.

GlgP affects glycogen structure by removing glucose units from the polysaccharide outer chains. The majority of characterized polyglucan phosphorylases are unable to act on chains smaller than five glucose residues in length (12, 50), the same behavior being found in *E. coli* GlgP (see Table S1 in the supplemental material). According to the generally assumed model of glycogen degradation (4), GlgX would be responsible for cleaving the small maltotretraose stubs produced by GlgP, thereby terminating glycogen breakdown. Both the glycogen excess phenotype and the altered structure of the polysaccharide in *E. coli glgX* deletion mutants (24) are certainly consistent with this view. In order to study the impact of GlgP absence on the glycogen structure in vivo, we analyzed the degree of polymerization (DP) of glycogen isolated from WT and Δ*glgP* cells. The results displayed in Fig. 4 clearly show an increase in chains corresponding to a DP of 13 to 30 glucose residues at the expense of chains of six, seven, and eight residues in Δ*glgP* cells, which is consistent with a function of GlgP in the degradation of the polysaccharide outer chains. These chains would then be elongated by GlgA without being removed by the missing phosphorylase, thereby leading to the observed CLD.

To further prove that the absence of GlgP has the largest impact on the polysaccharide's outer chains, glycogens from WT and Δ*glgP* cells were subjected to digestion with β-amylase, an exo type of hydrolase that produces β-maltose by digesting the outer chains of glycogen, amylose, and amylopectin. This enzyme is unable to bypass or hydrolyze the α-1,6 linkages and therefore stops two, three, or four residues from

the first α-1,6 branch encountered to generate the so-called β-limit dextrin. If the dramatic differences seen in Fig. 4 were essentially confined to the outer chains of the polysaccharide, then the β-limit dextrins obtained from glycogen from WT and Δ*glgP* cells would have similar CLDs. Results displayed in Fig. 5 clearly show that this is the case, thereby proving that GlgP catalyzes glycogen breakdown by removing glucose units from the polysaccharide outer chains in *E. coli*.

Regulatory considerations of GlgP. We have confirmed that changes in the glycogen content in both Δ*glgP*- and *glgP*-overexpressing cells grown in the presence of glucose are due to changes in glycogen phosphorylase activities and not to pleiotropic changes in the activities of enzymes closely linked to glycogen biosynthesis. As shown in Table 3, GlgC, GlgA, PGM, and AspP activities in both Δ*glgP*- and *glgP*-overexpressing cells did not vary much with respect to those of WT cells. Remarkably, these analyses also revealed that maximum catalytic glycogen phosphorylase activities in WT cells are higher than those of gluconeogenic enzymes, such as GlgC and GlgA, during glycogen accumulation. This, and the fact that a moderate increase of glycogen phosphorylase activity leads to a dramatic reduction of glycogen content (Fig. 3B), indicates that, unless GlgP is highly regulated in the bacterial cell, glycogen accumulation would be prevented. In this context, it is noteworthy that, in contrast to the highly regulated eukaryotic glucan phosphorylases, bacterial phosphorylases show no apparent regulatory properties and are thought to be exclusively controlled at the level of gene expression (12, 36, 42, 49, 53). However, several reports have suggested that bacterial GlgPs may be subjected to posttranslational regulation. Chen and Segel (19), for instance, showed that *E. coli* glycogen phosphorylase activity in the glycogen synthetic direction can be moderately activated by AMP and inhibited by ADPG. However, our results indicate that GlgP does not exhibit regulatory properties with respect to substrates of physiological relevance, such as AMP, ADPG, UDP-glucose, glucose, and hexose-Ps, when assayed in the glycogen breakdown direction (see Table S2 in the supplemental material). GlgP could also be allosterically modulated in *E. coli* as a result of its interaction with the histidine phosphocarrier protein (HPr) of the phosphotransferase system (51). Other models of bacterial GlgP regulation are based on the occurrence of both particle-associated and soluble GlgP states, whose balance has been proposed to control glycogen accumulation (45). Needless to say, possible regulatory properties of GlgP and its importance in the control of glycogen accumulation need to be further investigated.

TABLE 3. Activities of some enzymes closely linked to glycogen metabolism in transformed BL21(DE3)C43 cells[a]

Enzyme	Activity (mU/mg of bacterial protein) in cells transformed with:		
	pET-15b	Δ*glgP*	pET-glgP
GlgC	2.0	2.3	1.8
GlgA	1.5	1.7	1.6
GlgP	9.8	BDL[b]	209
PGM	205	96	160
AspP	9.3	8.9	10

[a] BL21(DE3)C43 cells transformed with either pET-15b or pET-glgP and Δ*glgP* cells entering the stationary phase were assayed.
[b] BDL, below the detection limit.

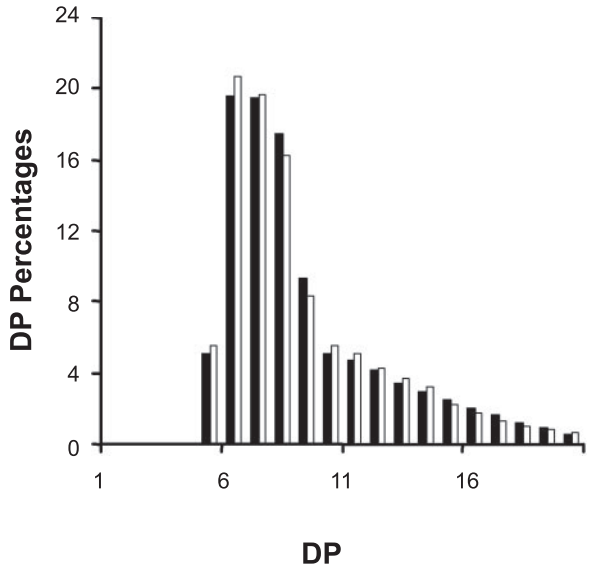

FIG. 5. CLD of glycogen β-limit dextrins determined by FACE. Isoamylase debranched glycogen β-limit dextrins from both WT and Δ*glgP* cells were produced as described in Materials and Methods and separated up to DP 20. Chains below DP 5 are not represented because of the very high background due to the presence of hyperabundant chains of DP 2 and 3 generated by the action of β-amylase. Dark bars correspond to WT cells, and light bars correspond to Δ*glgP* cells. The bacterial strain used in this study was TG1. Essentially similar results were obtained with other bacterial strains.

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