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Biochemistry

II-B^{Glc}, a glucose receptor of the bacterial phosphotransferase system: Molecular cloning of *ptsG* and purification of the receptor from an overproducing strain of *Escherichia coli*

(membrane protein/phospho*enol*pyruvate:glycose phosphotransferase system-mediated repression/octylpolyoxyethylene/hydroxylapatite chromatography/methyl α-glucoside transport)

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ABSTRACT **The bacterial phospho*enol*pyruvate:glycose phosphotransferase system (PTS) consists of interacting cytoplasmic and membrane proteins that catalyze the phosphorylation and translocation of sugar substrates across the cell membrane. One PTS protein, II-B^{Glc}, is the membrane receptor specific for glucose and methyl D-glucopyranosides; the protein has been purified to homogeneity from *Salmonella typhimurium* [Erni, B., Trachsel, H., Postma, P. & Rosenbusch, J. (1982) *J. Biol. Chem.* 257, 13726–13730]. In the present experiments, the *Escherichia coli ptsG* locus, which encodes II-B^{Glc}, was isolated from a transducing phage library and subcloned into plasmid vectors. The resulting plasmids complement the following phenotypic defects of *ptsG* mutants: growth on glucose, uptake and phosphorylation of methyl α-D-glucoside, and repression of the utilization of non-PTS sugars by methyl α-glucoside. The transformed cells overproduce II-B^{Glc} 4- to 10-fold, and a M_r 43,000 polypeptide was synthesized from the plasmids in an *in vitro* transcription/translation system. The *E. coli* and *S. typhimurium* II-B^{Glc} proteins differ in their physical properties, and a modified, three-step purification procedure was developed for isolating the *E. coli* protein.**

The bacterial phospho*enol*pyruvate:glycose phosphotransferase system (PTS) comprises interacting cytoplasmic and integral membrane proteins that participate in several metabolic and regulatory processes within the cell (for recent reviews see refs. 1 and 2). One important function of the PTS is to catalyze the group translocation of its sugar substrates (PTS sugars). The phosphate group from intracellular phospho*enol*pyruvate (*P-enol*Prv) is sequentially transferred to enzyme I of the PTS (enzyme I), to histidine-containing phosphocarrier protein of the PTS (HPr), to a set of sugar-specific proteins, and finally to the sugar substrate as it is translocated across the membrane. Enzyme I and HPr, which are cytoplasmic proteins, are required as general phosphocarrier proteins for the transport and phosphorylation of all PTS sugars. Sugar specificity is determined by enzyme II complexes, which are primarily membrane proteins.

Glucose is translocated in enteric bacteria by two enzyme II complexes, II^{Glc} and II^{Man} (3). The II^{Man} complex consists of lipid and at least two integral membrane proteins, II-A^{Man} and II-B^{Man} (4–7), and phosphorylates mannose, glucose, and their analogues. The II^{Glc} complex is more specific in its substrate requirements [glucose and methyl α- and β-D-glucopyranoside (α- and βMeGlc)] and also comprises two proteins: a cytoplasmic protein, III^{Glc} (the glucose-specific phosphocarrier protein of the PTS), and the membrane receptor II-B^{Glc} (the glucose-specific integral membrane protein of the PTS). The II^{Man} and II^{Glc} complexes are kinetically distinguishable *in vivo* and *in vitro* (8).

Understanding the various functions catalyzed by the PTS requires a detailed analysis of how the proteins, lipid, and ligands interact. In the first such experiments, with enzyme I from *Salmonella typhimurium* (9), the limited quantity of protein available from wild-type cells greatly restricted the work. We therefore turned to *Escherichia coli* strains that overproduce the desired protein. The *E. coli* genes that encode enzyme I, HPr, and III^{Glc} have been cloned and sequenced (10–12), and the cloning of genes encoding the II^{Man} complex (the *ptsM* locus) has recently been completed (5–7).

The present experiments are concerned with II-B^{Glc}, the membrane component of the II^{Glc} complex. Mutations affecting the expression and activity of II-B^{Glc} in *S. typhimurium* and *E. coli* map to the *ptsG* locus (3, 8). We describe the molecular cloning of the *E. coli ptsG* locus and the construction of II-B^{Glc} overproducing strains. The protein purified from *S. typhimurium* membranes (13, 14) exhibits different physical properties from the *E. coli* protein; a modified procedure was therefore developed for purification of the *E. coli* enzyme. A preliminary report has been presented (15).

MATERIALS AND METHODS

Materials. [^{14}C]αMeGlc was synthesized by a modification of the method of Bollenback (16) using [U-^{14}C]glucose (specific activity, 12,000 GBq/mol; Amersham) and purified by the method of Austin *et al.* (17). The detergent octylpolyoxyethylene (octyl-POE) (18) was essential for the purification of II-B^{Glc} from the membranes and was generously supplied by J. P. Rosenbusch (Biozentrum, University of Basel, Department of Microbiology, CH-4056 Basel, Switzerland).

Isolation of a *ptsG*$^+$ Transducing Phage. The genotypes of bacterial strains and phages used in these studies are listed in Table 1. Three λ:*E. coli* hybrid transducing phage libraries were screened for *ptsG*$^+$. *E. coli* SR423 was the recipient for hybrid phage stocks A and B; SR1210 was the recipient for pool E. Recipient cells were infected with phage, plated on medium A agar (23) with 1% glucose, and incubated at 30°C. Single colonies, streaked on Difco MacConkey agar (1% glucose), were scored for glucose fermentation and phage release appropriate to *imm*^{21} lysogens. Uptake of [^{14}C]αMeGlc (24) was also used as a criterion for restoration of

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Abbreviations: PTS, phospho*enol*pyruvate:glycose phosphotransferase system; *P-enol*Prv, phospho*enol*pyruvate; enzyme I, enzyme I of the PTS; HPr, histidine-containing phosphocarrier protein of the PTS; αMeGlc, methyl α-D-glucopyranoside; II-B^{Glc}, glucose-specific integral membrane protein of the PTS; III^{Glc}, glucose-specific phosphocarrier protein of the PTS; Glc$^+$, capable of glucose fermentation; Amp^r, ampicillin resistant; octyl-POE, octylpolyoxyethylene.

Table 1. Bacterial and phage strains

Strain	Genotype	Source or ref.
S. typhimurium		
PP1133		19
E. coli		
1100	K-12 derivative	20
SK1592	*gal thi* T1^r *endA hsdR sbcB*	21
SR423	SK1592 *ptsM ptsG*	C. Shea*
SR425	SK1592 *ptsM ptsG*	C. Shea*
JM1100	*ptsM ptsG ptsF galK* DE79 *galP*	22
ZSC113	*ptsM ptsG glk lacZ rha rpsL relA*	3
SR1202	ZSC113:pBR322	This study
SR1210	SR423::λ202	This study
SR1216	SR423::λE-11	This study
SR1301	ZSC113:pCB10	This study
SR1307	ZSC113:pCB26	This study
SR1305	ZSC113:pCB20	This study
SR1308	ZSC113:pCB30	This study
Phage		
λ202	λ*imm*^{21}Δ*b*515Δ*b*519	M. Pearson†
λ:*E. coli* A	λKK2:*E. coli* KL16 (*Hin*dIII)	S. Rogers‡
λ:*E. coli* B	λNM816:*E. coli* KL16 (*Eco*RI)	S. Rogers‡
λ:*E. coli* E	λKK2:*E. coli mutD* (*Xho* I)	S. Rogers‡

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ptsG$^+$ function. Phage lysates, prepared from *imm*^{21} lysogens capable of glucose fermentation (Glc$^+$) (25), were tested in SR1210 for the presence of *ptsG*$^+$ transducing phage. The resulting *imm*^{21} lysogens were tested for *ptsG*$^+$ as described above.

Subcloning the Transducing Phage DNA. DNA was extracted from CsCl gradient-purified phage fractions (25), digested with several restriction enzymes, including *Xho* I, *Bam*HI, *Hin*dIII, *Sal* I, *Pst* I, and *Eco*RI (Bethesda Research Laboratories), and ligated into the appropriate site of pBR322 with T4 DNA ligase (Bethesda Research Laboratories). Competent bacteria were transformed by the procedure of Mandel and Higa (26), selecting for transformed cells on MacConkey agar (1% glucose) and ampicillin (30 μg/ml). The transformants were screened for glucose fermentation and *ptsG*$^+$ activity. Small-scale plasmid preparations (27) were tested for ability to retransform the mutant strain to Glc$^+$ ampicillin resistant (Amp^r). When transcriptional induction was desired in transformants containing a derivative of pDR720, 3-β-indoleacrylic acid (Sigma) was added at a final concentration of 5 μg/ml to cells 1 hr prior to harvest (28).

Isolation of *ptsG*$^+$ from Genomic DNA. Genomic DNA, isolated from *E. coli* 1100 as described (29), was digested to completion with *Sal* I. Fragments 2.3–4.4 kilobases (kb) in length, extracted from a low-melting temperature agarose gel using an Elutip-d DNA purification minicolumn (Schleicher & Schuell), were ligated into pBR322 or pDR720 (Pharmacia). Transformation, screening, and small-scale plasmid isolations were conducted as described above.

Phenotypic Properties of Transformed Cells. Several *E. coli ptsG* mutants and their transformants harboring various plasmids were tested for growth in medium A (23) supplemented with ampicillin (30 μg/ml), Casamino acids (0.1%), and glucose (0.2%), maltose (0.2%), glycerol (0.2%), or lactose (0.2%). The cultures were grown at 37°C and optical density was measured at 600 nm. Doubling times were determined from the slope of the growth curve during logarithmic-phase growth.

The same strains were tested for repression of growth on non-PTS sugars in the presence of αMeGlc or 2-deoxyglucose. Cells were grown on medium A as described above. When the cultures reached an optical density of ≈0.15, αMeGlc (Aldrich, 99%) or 2-deoxyglucose (Sigma, grade III) was added to a final concentration of 10 mM.

Transport experiments were conducted with *E. coli* 1100, SR1305, SR1308, and ZSC113 cells using [^{14}C]αMeGlc (50 μM) as described by Stock *et al.* (8). The cells were grown in minimal medium A supplemented with glucose (0.2%) as described above.

***In Vitro* Transcription/Translation of Plasmid-Encoded Proteins.** Covalently closed circular plasmids (pBR322, pDR720, pCB10, pCB20, pCB26, and pCB30) were used as templates for a prokaryotic coupled transcription/translation system as directed by the manufacturer (Amersham). Trichloroacetic acid-precipitable products were separated by NaDodSO$_4$/PAGE (10% acrylamide/2.5% bisacrylamide), and [^{35}S]methionine-labeled proteins were visualized by autoradiography.

Preparation of Membranes for the Assay of II-B^{Glc}. Cells were grown in medium A containing 0.2% Casamino acids, 0.5% glucose, and 30 μg of ampicillin per ml and were harvested at late exponential phase of growth; the membranes were prepared as described (30).

Assay for the Activity of II-B^{Glc}. The published procedure (30) was employed with the addition of bovine serum albumin (crystalline, 125 μg) and L-α-dioleoylphosphatidyl-DL-glycerol (Sigma) (75 μg) to the 0.1-ml assay mixture. Enzyme I (2 units) (23), HPr (3 μM) (31), and III$^{Glc}_{Slow}$ (1–25 μM) (32) were homogeneous. Sugar phosphate formation was directly proportional to the quantity of II-B^{Glc} and to the time of incubation for at least 45 min. A unit of II-B^{Glc} activity is defined as the quantity of enzyme catalyzing the production of 1 μmol of sugar phosphate in 30 min; specific activity is defined as units/mg of protein.

Purification of II-B^{Glc} from *S. typhimurium* and *E. coli*. Details of the purification procedures will be presented elsewhere. For the enzyme from *S. typhimurium*, the method of Erni *et al.* (13) was followed through the isoelectric focusing step. At this stage, the fractions containing II-B^{Glc} were chromatographed on a calibrated Sephadex G-50 column; this was followed by fractionation on a chromatofocusing column (13). A new step was introduced at this point, chromatography on a hydroxylapatite (Calbiochem–Behring, fast-flow grade) column (33). For purification of II-B^{Glc} from the overproducing strain of *E. coli*, SR1308, the procedure was modified. In a typical preparation, 10 g (wet weight) of cells was ruptured, the membranes were extracted by using octyl-POE, and purification was effected by the following sequence of steps: isoelectric focusing, Sephadex G-50 filtration, and adsorption and elution from the hydroxylapatite column.

Protein concentration was measured by using published methods (34, 35).

RESULTS

Cloning the *E. coli ptsG* Gene. Three λ:*E. coli* hybrid phage pools were screened for *ptsG*$^+$ in a *ptsG ptsM* host strain, using glucose fermentation and αMeGlc uptake (24) as criteria for restoration of *ptsG*$^+$ function. Only phage pool E, containing *Xho* I fragments of *E. coli* DNA, produced Glc$^+$ lysogens in SR1210. Phage lysates made from six isolates were capable of transducing *ptsG*$^+$ into SR1210, demonstrating that the original isolates carried an inducible *ptsG*$^+$ prophage. Transducing phage were prepared on a large scale from SR1216, and the DNA was isolated from phage fractions that produced Glc$^+$ lysogens in SR1210. The phage DNA was digested with various restriction enzymes and subcloned into pBR322. *E. coli* SR423, SR425, JM1100, and ZSC113 were transformed to Glc$^+$ Amp^r by plasmids containing a 3.3-kb *Sal* I fragment of phage DNA. Restriction maps of these plasmids (pCB10, pCB11) are shown in Fig. 1. The 3.3-kb

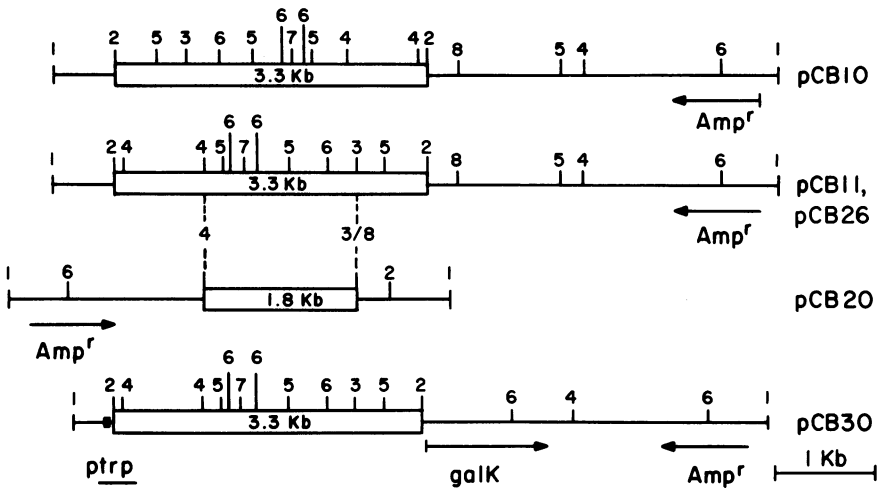

FIG. 1. Restriction endonuclease maps of plasmids carrying the *ptsG* gene. The thin lines represent the plasmid vector and the rectangles represent *ptsG* transducing phage DNA or *E. coli* genomic DNA. The plasmids pCB10 and pCB11 are pBR322 derivatives containing a 3.3-kb fragment of *ptsG* transducing phage DNA; the plasmid pCB26, which contains a 3.3-kb fragment of *E. coli* genomic DNA, is identical, by restriction endonuclease analysis, to pCB11. The 1.8-kb fragment of pCB26 was inserted into pBR322 to construct pCB20. The plasmid pCB30 is a pDR720 derivative with the 3.3-kb transducing phage fragment under control of the *trp* promoter. The position of the *trp* promoter in pCB30 is indicated by the shaded square. Restriction sites are indicated by the following numbers: 1, *Eco*RI; 2, *Sal* I; 3, *Hpa* I; 4, *Nde* I; 5, *Pvu* II; 6, *Pvu* I; 7, *Nco* I; 8, *Nru* I.

fragment was also inserted into pDR720. The resulting plasmid, pCB30, contains the cloned sequences downstream from the inducible *trp* promoter-operator (Fig. 1). A genomic 3.3-kb *Sal* I fragment was isolated from *E. coli* 1100 (pCB26) that likewise complemented the *ptsG* defect in these strains. Restriction maps of this genomic *Sal* I fragment and the phage fragment were identical. In addition, *ptsG* mutants transformed by pCB20, a subclone of pCB26 (Fig. 1), were Glc$^+$ Amp^r, indicating that the 1.8-kb *Hpa* I–*Nde* I fragment carries the gene. Purified preparations of all plasmids transformed SR423, SR425, ZSC113, and JM1100 to Glc$^+$ Amp^r. All transformants that were Glc$^+$ on MacConkey glucose agar were able to take up [^{14}C]αMeGlc on LB agar.

Restoration of II-B^{Glc} Activity and Function to *ptsG* Mutant Strains. Though the transformants containing the cloned *ptsG* gene could ferment glucose and take up labeled αMeGlc, quantitative data were sought to establish whether II-B^{Glc} function had been fully restored to the mutants by the plasmids.

The transformants grew well on glucose as the sole carbon source; their generation times were about 1.1 hr, comparable to the generation time of wild-type strains in the same medium.

Another characteristic expected of *ptsG*$^+$ cells is PTS-mediated repression by αMeGlc—that is, αMeGlc should inhibit the cells from utilizing non-PTS sugars such as glycerol, maltose, melibiose, and lactose (1, 2). *PtsG* mutants transformed with the plasmids shown in Fig. 1 did exhibit this behavior with these substrates. Some results with glycerol and maltose are shown in Fig. 2. The control strains ZSC113 and SR1202 and the plasmid-containing transformants grew well on both non-PTS sugars. When αMeGlc was added to the medium, the control strains continued to grow normally, whereas the transformants exhibited an immediate growth stasis. The duration of the growth lag varied from 1 to 6 hr, even with the same strain. Furthermore, after the cells recovered from the stasis, they grew on the non-PTS compounds at a rate of 50% or less than the rate prior to addition of αMeGlc. Similar results were obtained with lactose in experiments employing a series of transformants derived from *E. coli* JM1100 (ZSC113 cannot utilize lactose).

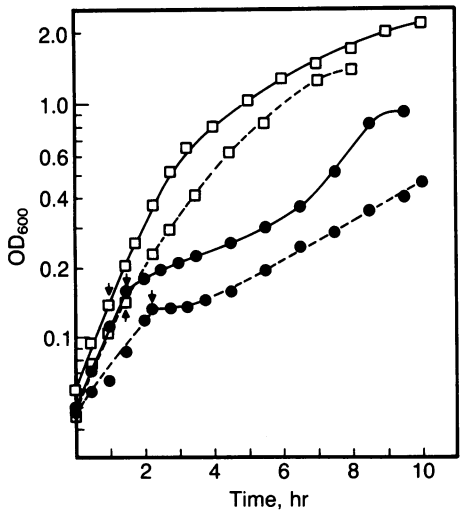

FIG. 2. Repression of growth on non-PTS sugars. Cells were grown in minimal medium A supplemented with 0.2% glycerol or maltose. αMeGlc was added to a final concentration of 10 mM at the point indicated by the arrow. □—□, SR1202, glycerol; □- -□, SR1202, maltose; ●—●, SR1301, glycerol; ● - - ●, SR1301, maltose.

Neither the control strains nor the transformants showed PTS-mediated repression in the presence of 2-deoxyglucose (data not shown), an analogue that is taken up by the complex encoded by *ptsM*. The specificity of the response to αMeGlc therefore indicates that the cloned DNA fragments carry *ptsG*$^+$.

Transformation to *ptsG*$^+$ should restore the ability of membranes to phosphorylate αMeGlc *in vitro*. Table 2 gives the results of II-B^{Glc} assays with membrane preparations from different strains. Membranes derived from II-B^{Glc} mutants containing the *ptsG*$^+$ transducing phage or any of the plasmids shown in Fig. 1 were able to phosphorylate αMeGlc. Furthermore, the specific activities of the different membranes were 4- to 10-fold higher than those from wild-type cells.

Transport Experiments. The results described above showed that the transformed cells produced II-B^{Glc} but only

Table 2. II-B^{Glc} activity in membranes isolated from *ptsG* mutants and transformed strains

Strain	II-B^{Glc} specific activity
1100	42
SR423	1
SR1216	178
ZSC113	0.4
SR1301	316
SR1307	470
SR1305	218
SR1308	300
SR1308*	350

Cell membranes were isolated and assayed for II-B^{Glc} activity. Specific activity is given in μmol of αMeGlc-*P* formed in 30 min/mg of membrane protein.

*3-β-Indoleacrylic acid (final concentration, 5 μg/ml) was added 1 hr prior to cell harvest.

suggested that the protein was properly inserted into the cell membrane. An improper orientation of the protein in the membrane might still permit growth on glucose and phosphorylation of αMeGlc by membrane preparations. The most significant assay for II-B^{Glc} function, therefore, is the transport and concomitant phosphorylation of αMeGlc by whole cells. The results of such experiments are shown in Fig. 3. The transport and phosphorylation of αMeGlc by *ptsG* mutants containing the cloned gene were comparable to those of wild-type cells.

All of the data lead to the conclusion that *ptsG* mutants harboring either the 3.3-kb *Sal* I or the 1.8-kb *Hpa* I–*Nde* I DNA fragment showed all of the properties of *ptsG*$^+$ cells.

Plasmid-Encoded Gene Products. Plasmid-encoded proteins were analyzed with the aid of an *in vitro* transcription/translation system. The resulting [^{35}S]methionine-labeled translation products, after separation by NaDodSO$_4$/PAGE, are shown in Fig. 4. In this system, pBR322 produced a labeled protein at M_r 30,000, corresponding to the processed form of β-lactamase. The other plasmid used as a vector, pDR720, gave a similar band (not shown). The plasmids containing the *ptsG*$^+$ gene (pCB10, pCB20, and pCB30) each produced an additional band at M_r 43,000, expected from the reported molecular weight of II-B^{Glc} (13).

Purification and Properties of II-B^{Glc} from *E. coli* and *S. typhimurium*. II-B^{Glc} has been purified from *S. typhimurium* (13); we first used this procedure with *S. typhimurium* PP1133 and *E. coli* SR1308. The method was not satisfactory for the *E. coli* enzyme, however, which has a much higher isoelectric point than the protein from *S. typhimurium* (approximately pH 9 and 6.5, respectively) and so does not bind to the chromatofocusing resin. A modified purification procedure was developed that gave the desired results, and the enzyme purified from both species of bacteria gave similar patterns on NaDodSO$_4$/PAGE to those reported (13).

The specific activity of the purified enzyme from *S. typhimurium* was found to be ≈30-fold higher than that reported previously—≈2000 units/mg of protein. Much of the difference can be explained by the use of higher concentrations of III^{Glc} in the present studies, but an additional factor is that activity is not lost at the final step of the present procedure (hydroxylapatite chromatography). The final preparation of the *E. coli* enzyme showed a higher specific activity than did the enzyme from *S. typhimurium* (2870 and 2030 units/mg of protein, respectively).

The purified proteins from the two species showed similar kinetic properties (K_m and V_{max}) with respect to the substrates αMeGlc, 2-deoxyglucose, and III^{Glc} and also with respect to their lipid requirements for activation. Furthermore, the K_m values of the crude and purified enzymes were found to be similar, an important result showing that the purification procedure did not alter the properties of the enzyme. The details of the purification and kinetic experiments will be presented elsewhere.

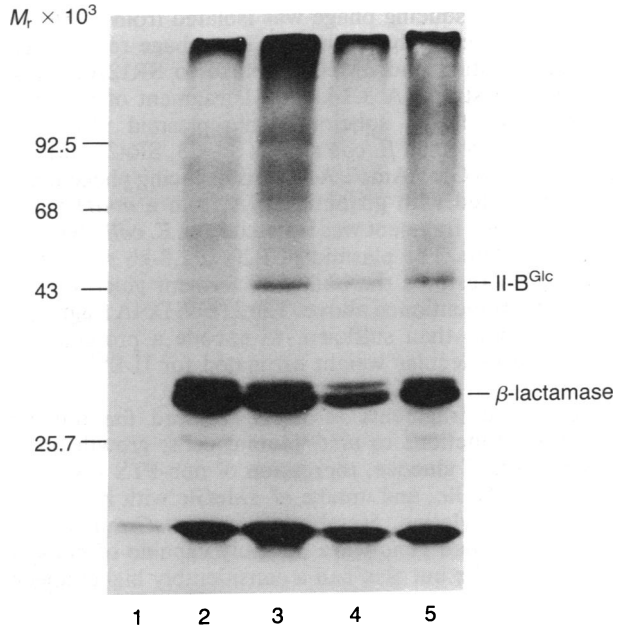

FIG. 4. Analysis of plasmid-encoded polypeptides. Circular plasmid DNA (1.5 μg) was used as a template for the synthesis of [^{35}S]methionine-labeled plasmid-encoded gene products. Trichloroacetic acid-precipitable translational products were separated by NaDodSO$_4$/PAGE and detected by autoradiography. Lane 1, no DNA (control); lane 2, pBR322; lane 3, pCB10; lane 4, pCB26; lane 5, pCB20. The molecular weights and gene products are indicated.

DISCUSSION

The enzyme II complexes are the sugar-specific membrane components of the PTS. These studies focus on II-B^{Glc}, the high-affinity glucose receptor of the PTS (1, 8). To date, the genetic loci encoding II^{Mtl} (*mtlA*) (36, 37) and the II^{Man} complex (*ptsM*) (5–7) are the only PTS membrane components that have been cloned from enteric bacteria. Here we report the molecular cloning of *ptsG*, the locus encoding II-B^{Glc}, from *E. coli*.

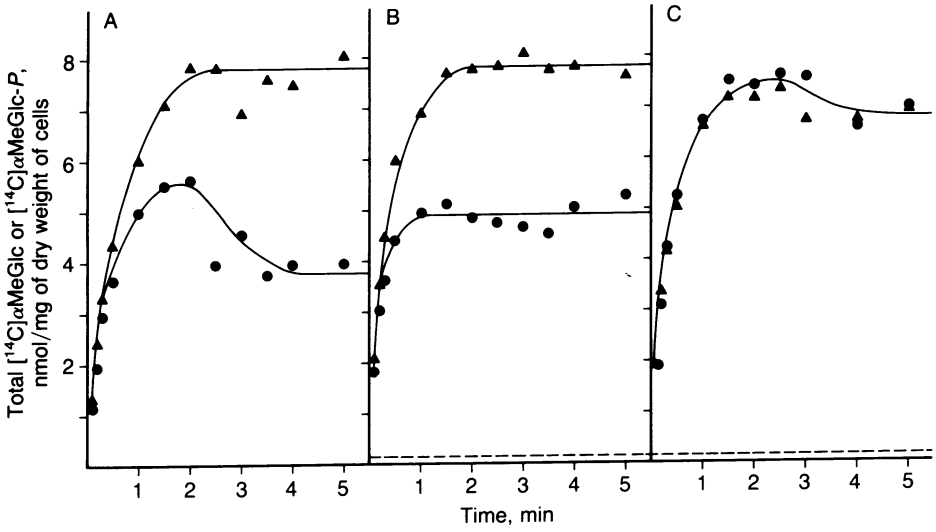

FIG. 3. αMeGlc transport by *E. coli* 1100, ZSC113, and transformants of ZSC113. Cells were grown in glucose minimal medium A to late logarithmic phase. Cells were harvested and αMeGlc transport was measured as described (8), using 50 μM αMeGlc. Total [^{14}C]αMeGlc (▲), which includes free and phosphorylated αMeGlc, and [^{14}C]αMeGlc-*P* (●) are indicated for each strain. (*A*) 1100. (*B*) SR1305. (*C*) SR1308. Transport by the control strain ZSC113 is indicated in *B* and *C* (– – –).

A *ptsG*$^+$ transducing phage was isolated from a λ:*E. coli* hybrid transducing phage library. This phage restored glucose fermentation and αMeGlc uptake to SR1210, a *ptsG ptsM* mutant strain. A 3.3-kb *Sal* I fragment of the transducing phage DNA, subcloned into plasmid pBR322 or pDR720, transformed *E. coli* strains SR423, SR425, JM1100, and ZSC113 to Glc$^+$ Amp^r. As the transducing phage library was constructed with genomic DNA from a *mutD* mutant strain, the same fragment was isolated from *E. coli* 1100 DNA (Fig. 1, pCB26). The plasmid pCB20, a 1.8-kb subclone of pCB26, also restores the ability to ferment glucose to the *ptsG* mutants mentioned above. The *E. coli* DNA fragment in pCB20 is more than sufficient to encode a protein of M_r 43,000, the molecular weight estimated for II-B^{Glc} (Fig. 4) (13).

The cloned fragments of DNA restored the following phenotypic functions to *ptsG* mutant cells: growth on and fermentation of glucose, repression of non-PTS sugar utilization by αMeGlc, and uptake of αMeGlc with its concomitant phosphorylation. Membranes from *ptsG* mutants harboring the cloned gene were not only capable of phosphorylating αMeGlc but also had a considerably higher specific activity than membranes from wild-type cells. The difference in specific activities between SR1216 and plasmid-containing strains can be interpreted as a copy-number effect. Only one copy of the cloned sequence is present within the lysogen, whereas multiple copies are maintained when the gene is present on a plasmid vector such as pBR322 or pDR720. Induction of SR1308 with 3-β-indoleacrylic acid resulted in variable enhancement of II-B^{Glc} activity. The lack of a dramatic increase in II-B^{Glc} activity upon induction might be expected because of the limited capacity of the membrane to accommodate a large excess of II-B^{Glc}, which may possibly be lethal.

The results reported here show that it will be possible to isolate large quantities of II-B^{Glc}, a prerequisite for a detailed analysis of its interactions with other PTS proteins, lipid, ligands, etc. II-B^{Glc} and several other PTS proteins are among the few bacterial proteins known to be up-regulated by growth on glucose (8). DNA sequencing experiments should provide the amino acid sequence required for biochemical work and additional information on the transcriptional and translational regulation of the synthesis of this interesting membrane protein.

Note Added in Proof. After submission of this manuscript, the molecular cloning of *ptsG* was reported (38). The restriction fragments containing the cloned gene appear to be identical in both publications.

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