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Nucleotide Sequence and Transcription Start Point of the Phosphoglycerate Transporter Gene of *Salmonella typhimurium*

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We identified the phosphoglycerate transporter gene of *Salmonella typhimurium* and its polypeptide product and determined the nucleotide sequence of the gene. The predicted translation product was a protein of 406 amino acid residues and was extremely hydrophobic, a feature that is consistent with its role in membrane transport. Hydropathy analysis suggested that there are eight transmembrane segments of at least 20 amino acid residues for the protein. The transcription start point was mapped to lie at position −44 relative to the putative translational initiation start point. Comparison of PgtP with UhpT and GlpT, the membrane-bound proteins involved in the transport of hexose-6-phosphate and glycerol-3-phosphate, respectively, revealed a very high degree of amino acid sequence similarity among them, reflecting not only similar structures and functions among these polypeptides but also a common evolutionary origin for them.

Phosphoenolpyruvate, 2-phosphoglycerate, and 3-phosphoglycerate (3-PG) are transported into *Salmonella typhimurium* via the inducible transport system *pgt* (14). Induction of transport occurs only when inducer is present extracellularly; no induction occurs in the absence of inducer, even though phosphoglycerates are present intracellularly at millimolar concentrations (14).

The *pgt* system has been cloned previously (11). In this report we describe the identification of the transporter gene *pgtP*, its polypeptide product and cellular location, the nucleotide sequence of the gene, and the transcription start point. Expression of the *pgtP* gene requires a functional *pgtA* gene. The nucleotide sequence of this gene, which encodes an activator protein, has been determined previously (21).

MATERIALS AND METHODS

Bacterial strains and phages. The bacterial strains used in this study were all *Escherichia coli* K-12 derivatives: BK9MDG (F^- *thi hsdR hsdM endB metC*) (13) and JM103 (*thi pro leu endA*). Phages M13mp18 and M13mp19 were used for gene sequencing.

Plasmids. The plasmids used in this study were derivatives of pBR322, pACYC184 (2), and pT7-1 or pT7-2 (17) and were constructed by standard methodologies. Plasmid pGP1-2 was a gift from S. Tabor and C. C. Richardson (Harvard Medical School, Boston, Mass.).

Media. The bacterial strains were grown in nutrient broth, YT, or medium E (18) containing 0.5% succinate or 0.4% 3-PG. When required, amino acids were added to final concentrations of 30 to 50 μg/ml. The following antibiotics were used at the indicated concentrations: ampicillin, 35 μg/ml; tetracycline, 15 μg/ml; chloramphenicol, 30 μg/ml.

Enzymes and chemicals. Restriction endonucleases and DNA enzymes were obtained from Bethesda Research Laboratories (Gaithersburg, Md.) and New England BioLabs, Inc. (Beverly, Mass.). All chemicals were reagent grade and were obtained from commercial sources.

Manipulations of DNA. Plasmid DNA was prepared from cleared lysates by CsCl-ethidium bromide centrifugation, as described by Davis et al. (4). The methods described by Maniatis et al. (12) were used for DNA manipulations.

Identification of gene products. The phage T7 RNA polymerase-T7 promoter coupled system of Tabor and Richardson (17) was used to identify gene products encoded by *pgtP*, with the exception that labeling with [^{35}S]methionine was done for 10 min instead of 5 min.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. For sodium dodecyl sulfate-polyacrylamide gel electrophoresis, 12% slab gels were used, and samples were boiled for 3 min prior to application. A series of cross-linked cytochrome *c*'s were used as molecular weight standards. Gels were run at 30 mA of constant current for 4 h, stained with Coomassie brilliant blue, treated with En^{3}Hance (New England Nuclear Corp., Boston, Mass.), dried, and exposed to X-ray film for autoradiography.

3-PG transport assays. Strain CSR603 harboring particular plasmids was grown at 37°C in minimal medium (medium E) that contained 0.5% succinate as a carbon source and that was supplemented with thiamine, threonine, leucine, proline, arginine, and the appropriate antibiotics. When growth reached the exponential phase, cells were collected by centrifugation and washed twice with and suspended in medium E to an optical density at 660 nm of 3.0. When induction of the *pgt* transport system was required, 0.2% 3-PG was added to exponentially growing cells, and the cells were harvested 2 h later.

3-PG transport was measured as follows. A portion (25 μl) of the cell suspension prepared as described above was incubated at 37°C for 2 min, when 1 μl of 250 mM glucose was added. Fifteen seconds later, 1 μl of 3-phospho[^{14}C]glycerate (2.3 mM; specific activity, 55 mCi/mM) was added, and incubation was continued for the desired time intervals. To terminate transport, the mixture was diluted with 2 ml of medium E. Cells were collected on cellulose acetate membranes (pore size, 0.45 μm; Schleicher & Schuell, Inc., Keene, N.H.) and washed once with 2 ml of medium E. Membranes were dried and counted in toluene-based Omnifluor (New England Nuclear) in a liquid scintillation counter.

Determination of cellular locations of *pgt* proteins. Cells (10

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ml) with [^{35}S]methionine-labeled, plasmid pJH587-encoded proteins synthesized in the T7 RNA polymerase-T7 promoter coupled system (17) were pelleted by centrifugation, suspended in 0.6 ml of 0.1 M Tris hydrochloride (pH 7.6), and subjected to sonication 4 times for 15 s each time, with a 1-min cooling interval between sonications. After low-speed centrifugation (4,000 rpm in a rotor [RC-5B; Ivan Sorvall, Inc., Norwalk, Conn.]) for 10 min to remove unbroken cells, the supernatant was layered on top of a 0.2-ml-thick cushion in a tube containing 0.25 M sucrose, 10 mM Tris hydrochloride, (pH 7.6), 50 mM KCl, 10 mM $MgCl_2$, and 0.01% L-methionine and subjected to high-speed centrifugation (37,000 rpm in an SW50.1 rotor) for 2 h. The membrane fraction was suspended in 0.2 ml of 0.1 M Tris hydrochloride (pH 7.6) containing 0.4 M NaCl and then subjected to centrifugation as described above. Washing of the membrane with the buffer containing 0.4 M NaCl was repeated once.

Transcription start point determination. The site of transcription initiation was determined by the primer extension method of Hu and Davidson (10). RNA was isolated from plasmid pJH6-harboring strain BK9MDG grown on minimal medium containing 3-PG as the sole carbon and energy source by the method of Chen et al. (3), with the modification that phenol replaced *m*-cresol. The RNA was hybridized to single-stranded M13mp18 carrying the 3.0-kilobase-pair (kbp) *Hin*dIII-*Pst*I fragment, and the RNA-DNA complex was used as a template for the extension by T4 DNA polymerase of the ^{32}P end-labeled hexadecameric primer 5′-TTCACCACACCCTTCA-3′ (corresponding to positions −157 to −142 in Fig. 2) that were annealed to it. The reaction mixture was subjected to polyacrylamide gel electrophoresis with a control from which RNA was omitted and in parallel with corresponding sequence ladders, as described previously (10).

RESULTS AND DISCUSSION

Subcloning. We previously described (11) the cloning of the phosphoglycerate transport system of *S. typhimurium* LT-2 into pBR322. The plasmid, pBR322-pgt2, which contained a 14.4-kbp insert in pBR322 at the *Bam*HI site, was found to be capable of conferring on *E. coli* K-12 the ability to transport 3-PG and to utilize 3-PG as the sole carbon and energy source, suggesting that the genes for the entire *pgt* system are contained in the 14.4-kbp insert (11). This plasmid was renamed pJH5.

Subcloning of the 14.4-kbp insert was undertaken to localize genes of the *pgt* system. A series of subclones was constructed from plasmid pJH5, and their ability to confer

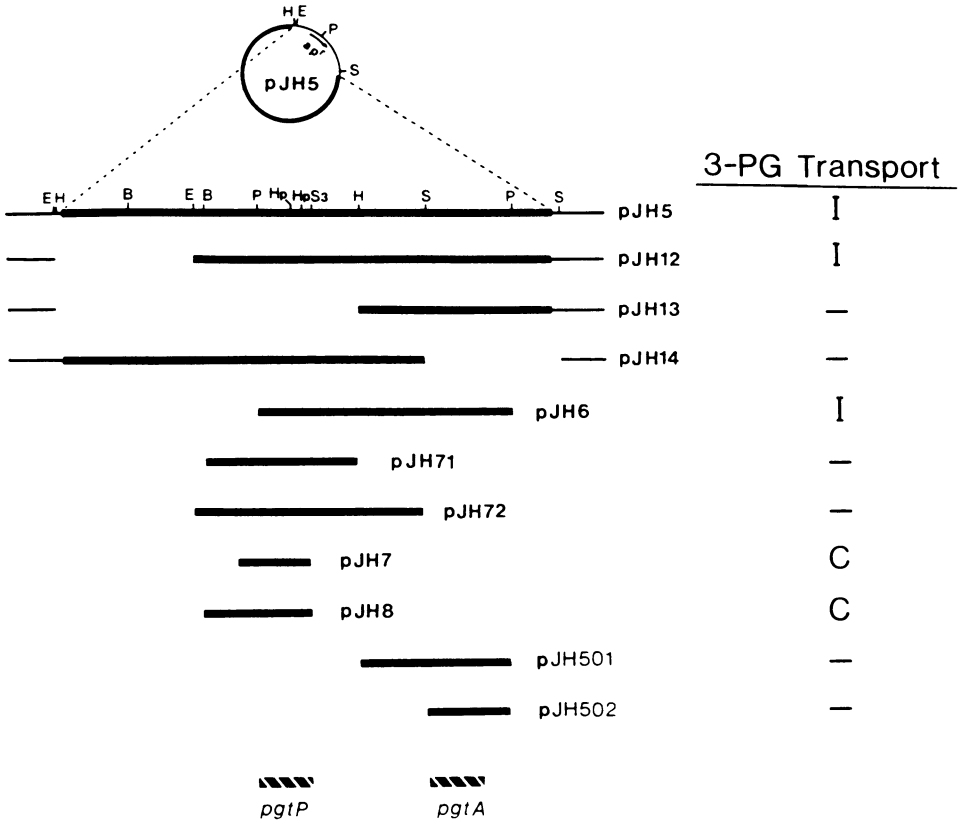

FIG. 1. Construction of *pgt* subclones from plasmid pJH5. Plasmids pJH12, pJH13, and pJH14 were derived from pJH5 by deleting *Eco*RI, *Hin*dIII, and *Sal*I fragments, respectively. Plasmid pJH6 was constructed by inserting the 7.6-kbp *Pst*I fragment into pBR322 at the *Pst*I site; plasmid pJH71 was constructed by inserting the 5.1-kbp *Bam*HI-*Hin*dIII fragment at the *Bam*HI and *Hin*dIII sites of pBR322; and pJH72 was constructed by inserting the 7.6-kbp *Eco*RI-*Sal*I fragment at the *Eco*RI and *Sal*I sites of pBR322. Plasmid pJH501 was constructed by cloning the 4.5-kbp *Hin*dIII-*Sal*I-*Pst*I fragment from pJH6 into pACYC184. A 2.6-kbp *Sal*I fragment was then removed from pJH501, to yield pJH502, which then contained only a 2.7-kbp *Sal*I-*Pst*I fragment of the original 7.6-kbp insert of pJH6. Construction of pJH7 and pJH8 are described in the text. Restriction sites are abbreviated as follows: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; P, *Pst*I; Hp, *Hpa*I; S, *Sal*I; S3, *Sau*3A. The broken bars indicate the locations of the *pgtP* and *pgtA* genes. Transport phenotypes are indicated as inducible (I), constitutive (C), or nonexpression (−).

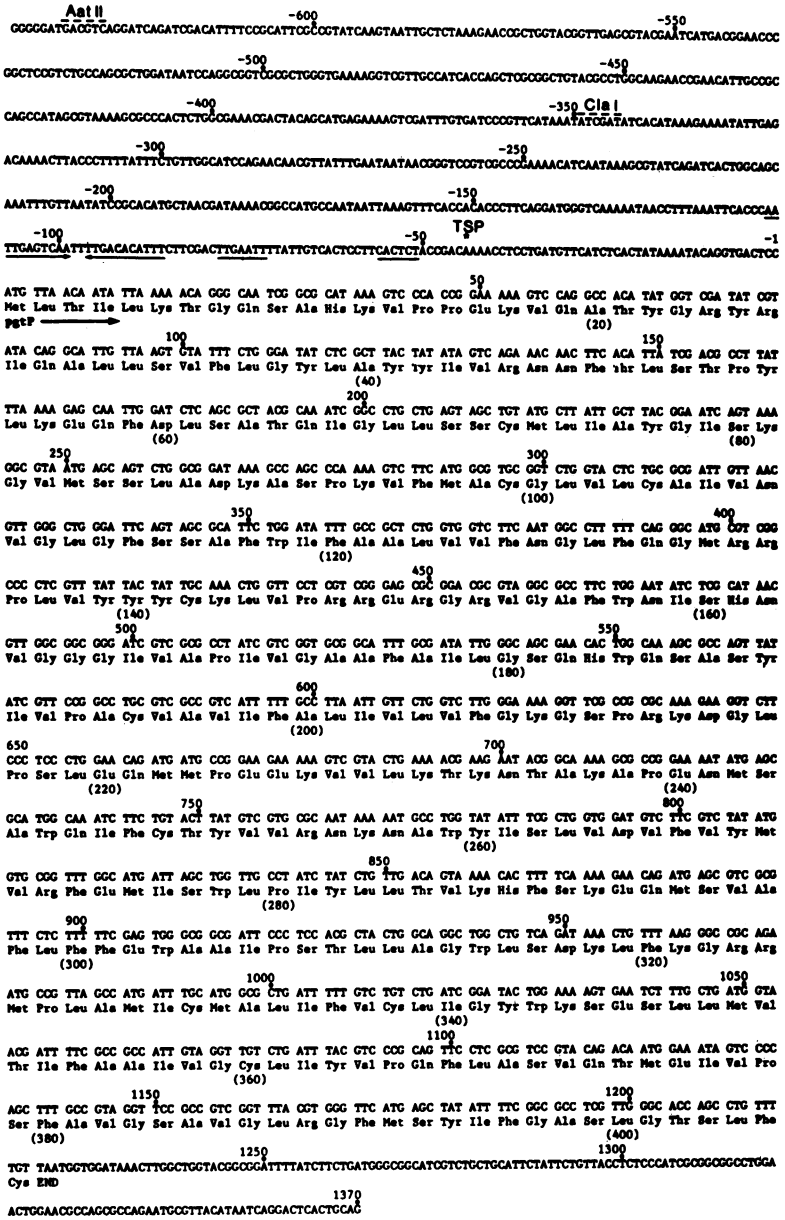

FIG. 2. The nucleotide sequences of the *pgtP* gene and its 5′-flanking region and the deduced amino acid sequence of PgtP polypeptide. The antisense (mRNA-like) strand is shown. The 3.0-kbp *Pst*I-*Hin*dIII and the 3.0-kbp *Pst*I-*Bgl*II fragments from pJH6 were cloned into M13mp19 at the *Pst*I and *Hin*dIII sites and the *Pst*I and *Bam*HI sites, respectively. A series of deletions from each clone was generated by the method of Henikoff (9). Nucleotide sequences were determined by the M13-dideoxynucleotide chain-termination method (15). A complementary universal 15-bp oligodeoxynucleotide was used as primer, and [^{35}S]dATP was used to label the products. Fractionation of the single-stranded DNA products of the primer elongation reaction was performed on 8% polyacrylamide gels. The numbers in parentheses indicate amino acid residues beginning from the N terminus. TSP indicates the transcription start point (see Fig. 3). The potential catabolite activator protein binding site is underlined with lines, with arrows, and the potential −10 and −35 promoter regions are indicated with thin lines.

3-PG transport was examined (Fig. 1). Deletion of the 3.3-kbp *Eco*RI fragment from the left arm of pJH5 yielding pJH12 had no effect on the inducible expression of 3-PG transport, indicating that this fragment contains none of the information needed for 3-PG transport or its regulation. However, deletion of the 9.0-kbp *Hin*dIII fragment from the left arm of pJH5, yielding pJH13, abolished 3-PG transport, indicating that the right half of the deleted fragment, namely, the 4.9-kbp *Eco*RI-*Hin*dIII fragment, contains information that is required for 3-PG transport, regulation, or both. Deletion of the 3.9-kbp *Sal*I fragment from the right arm of pJH5, yielding pJH14, also abolished 3-PG transport. When the 7.6-kbp *Pst*I fragment was subcloned into pBR322 at the *Pst*I site, the resulting clone conferred inducible 3-PG transport. However, subclones with inserts containing less than the full complement in pJH6, such as pJH71, pJH72, pJH501, or pJH502, conferred no 3-PG transport. Thus, it is evident that the genetic information necessary for inducible expression of 3-PG transport is contained in a 7.6-kbp *Pst*I fragment. We have previously identified (21) a regulatory

gene that is needed for the expression of 3-PG transport in this region; this gene, *pgtA*, encodes an activator protein and is located on the right arm of the 7.6-kbp *Pst*I fragment. Constitutive transport conferred by pJH7 and pJH8 (see below) suggests that a regulatory sequence is localized in the region of *Sau*3A-*Hin*dIII-*Sal*I-*Pst*I.

Localization of the transporter gene *pgtP*. To localize the structural gene(s) for the 3-PG transporter *pgtP*, subclones that were capable of conferring constitutive expression of 3-PG transport were sought. For this purpose, plasmid pJH5 was partially digested with *Sau*3AI. After electrophoresis fragments of 2 to 3 kbp in length were purified and ligated to pBR322 at the *Bam*HI site. The ligation mixture was then used to transform strain BK9MDG, and transformants that were able to utilize 3-PG as a source of carbon and energy were selected. Several of the 3-PG$^+$ clones were grown on minimal succinate medium in the absence of inducer (3-PG) and assayed for their ability to transport 3-PG. The two smallest plasmids, pJH7 and pJH8, which contained 2.2- and 3.3-kbp inserts, respectively, conferred a constitutive 3-PG transport ability (Fig. 1). Restriction analysis indicated that the inserts in these two plasmids correspond to the 1.6-kbp left arm of the 7.6-kbp insert in pJH6, as shown in Fig. 1, plus a short segment contiguous to its left end. For pJH7 this segment was 0.6 kbp, and for pJH8 it was 1.7 kbp. Thus, the structural gene(s) for the 3-PG transporter *pgtP* is contained within the 1.6-kbp *Pst*I-*Hpa*I-*Hpa*I-*Sau*3AI sequence. The sequences to the left of the 1.6-kbp arm are not present in pJH6 and are therefore not required for *pgt* expression. The location of the *pgtA* gene reported previously (21) is also indicated in Fig. 1. Located between the *pgtP* and *pgtA* genes are two *pgt* genes that are involved in the induction process of the *pgtP* gene expression (unpublished data). Sequencing of these genes is in progress.

Identification of the gene product. To identify the *pgtP* gene product that is encoded in the insert of plasmid pJH7 and the transcriptional direction of the gene, the 1.8-kbp *Pst*I-*Hin*dIII fragment of pJH7 was placed behind the phage T7 promoter of plasmids pT7-1 and pT7-2 at the *Pst*I and *Hin*dIII sites, generating pJH586 and pJH587, respectively, and the plasmid-encoded products were identified by the T7 RNA polymerase-T7 promoter coupled system of Tabor and Richardson (17). Plasmid pJH587, which carried the insert with the T7 promoter proximal to the *Hin*dIII site, encoded a rather diffused, 37-kilodalton product, but no product was observed with pJH586, which carried the insert in the opposite orientation (data not shown). Thus, it is evident that the 37-kilodalton product is the 3-PG transporter encoded by *pgtP* and that the direction of transcription of the *pgtP* gene is from right to left in Fig. 1.

The product of the *pgtP* gene is membrane bound. To determine the cellular location of the *pgtP* gene product, cells carrying plasmid pJH587 were labeled with [^{35}S]methionine in the T7 RNA polymerase-T7 promoter coupled system as described above, sonicated, and centrifuged to separate the membrane fraction from the soluble fraction. The transporter expressed by pJH587 was found to be associated with the membrane fraction, whereas the mature periplasmic β-lactamase was found in the soluble fraction, as expected (data not shown). Repeated washing of the membranes with buffer containing 0.4 M NaCl did not dissociate the proteins from the membranes (data not shown).

Nucleotide sequence of *pgtP* gene. The entire nucleotide sequence of the 3.0-kbp *Pst*I-*Hin*dIII fragment containing the *pgtP* gene was determined by using the M13-dideoxynucleotide chain-termination method (15). Both strands were sequenced. The sequences of the *pgtP* gene and its flanks are presented in Fig. 2. The *pgtP* gene is encoded in the sequence from positions 1 to 1218 with 406 amino acid residues. A putative Shine-Dalgarno sequence AGGTG at −10 to −6 precedes the coding frame. Sequences at positions −56 to −51 (5′-CACTCT) and −78 to −72 (5′-TTGAATT) are potential −10 and −35 promoter regions. At 10 bp upstream from these sequences (positions −106 to −89) is the 18-bp sequence 5′-TGAGTCAATTTTGACACA-3′, which is a potential catabolite activator protein-binding site.

Transcription start point. The transcription start point of the *pgtP* gene was determined by the examination of RNA transcripts by hybridization mapping of mRNA isolated from the pJH6-carrying strain grown on 3-PG. We used T4 DNA polymerase to extend a radiolabeled primer annealed to a single-stranded DNA template in the presence of mRNA (10). Because of the inability of T4 DNA polymerase to displace a RNA hybridized to DNA, primer extension should stop at the 5′ terminus of the hybridized mRNA, with the 3′ end of the growing DNA chain thereby marking its position. The site of the first termination of primer extension in the presence of mRNA was at position −44, whereas no termination was observed in that region in the absence of added mRNA (Fig. 3). Thus, the transcription start point of

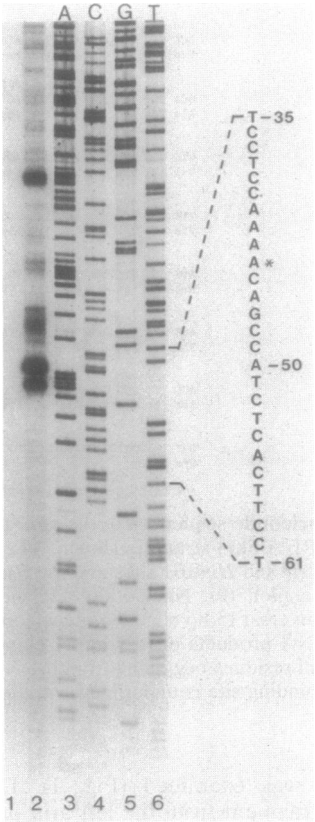

FIG. 3. Mapping of the transcription start point of the *pgtP* gene. The transcription start point was identified by primer extension analysis with T4 DNA polymerase, as described in the text. Lane 1, Primer extension in the absence of mRNA; lane 2, primer extension in the presence of mRNA; lanes 3 to 6, sequence ladders made by the dideoxynucleotide sequencing method with the same primer (unlabeled). Part of the nucleotide sequence deduced from the sequencing lanes is shown on the right, and the shortest extended primer segment is indicated with an asterisk.

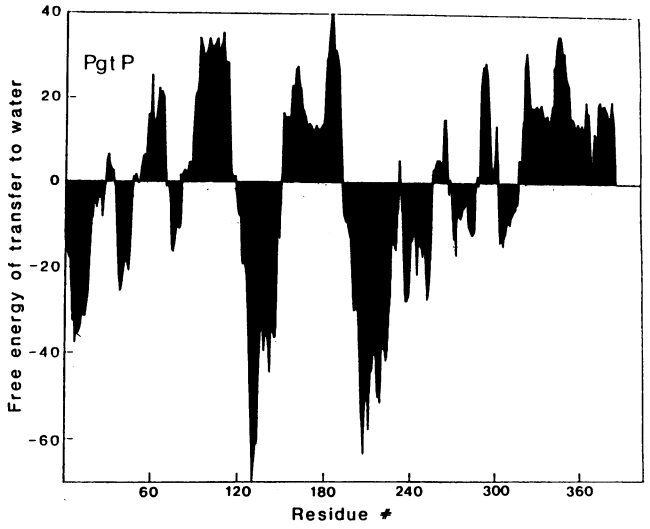

FIG. 4. Analysis of the 3-PG transporter amino acid sequence for potential transmembrane regions. This was determined by the method of Engelman et al. (6) by using a window of 20 amino acids. The potential transmembrane hydrophobic regions are above the horizontal zero line, whereas regions with a relatively hydrophilic nature are below the zero line. The amino acid numbers are the same as those in Fig. 2.

pgtP probably lies at position −44 relative to the translational initiation start point. Significant primer extension occurred beyond position −44, probably because of the existence of partially degraded mRNA molecules and possibly because of limited mRNA displacement by T4 DNA polymerase.

Amino acid sequence and protein structure. Examination of the deduced amino acid sequence of the PgtP polypeptide indicates that the polypeptide is extremely hydrophobic, because 67% of its total amino acid residues contain nonpolar side chains. The PgtP protein appears to be highly positively charged, with a total of 35 arginine plus lysine residues but only 17 aspartic plus glutamic acid residues. The calculated M_r of 44,800 for PgtP is larger than the apparent M_r of 37,000 observed on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Such anomalous behavior can be attributed to the high degree of hydrophobicity of the protein and is apparently characteristic of hydrophobic membrane transport proteins, such as the lactose permease (1) and glucose-6-phosphate carrier of *E. coli* (7, 19). Examination of the PgtP protein for potential transmembrane regions by the method of Engelman et al. (6) indicated the presence of eight such regions (Fig. 4), suggesting that the polypeptide traverses the membrane 8 times. The amino

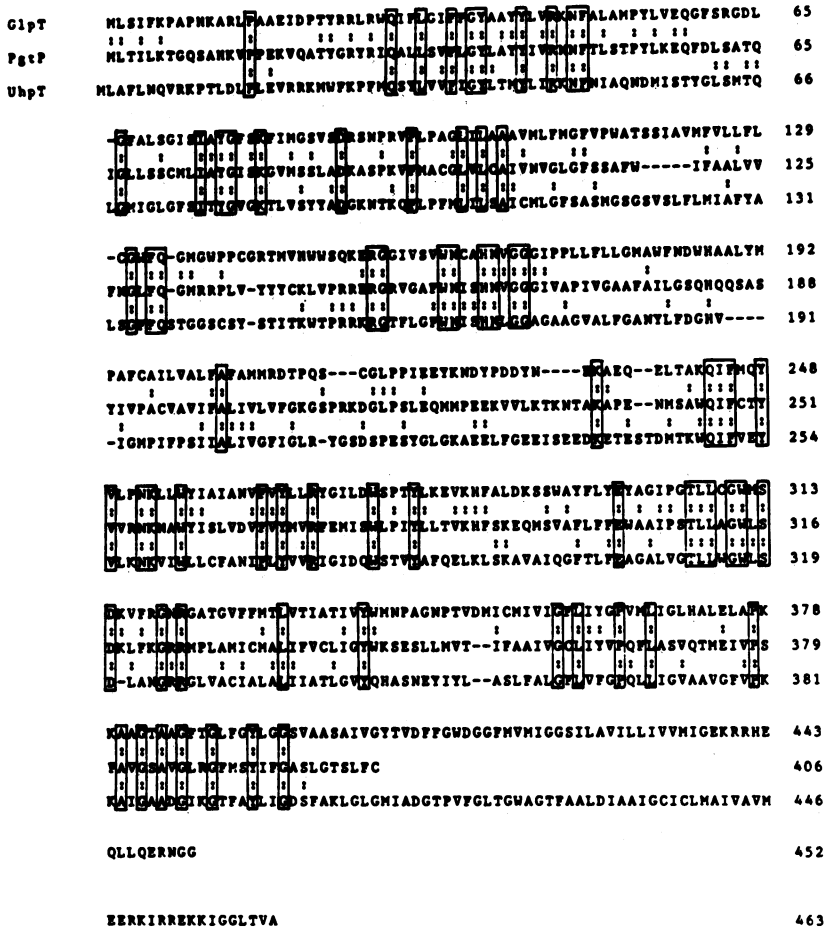

FIG. 5. Alignment of amino acid sequences of PgtP, UhpT, and GlpT polypeptides. Identical positions between a pair of polypeptides are indicated by double dots, and those among the three polypeptides are boxed. Gaps were introduced to optimize the alignment, which was generated as described by Wilbur and Lipman (20).

terminus, which is relatively hydrophilic in composition (Fig. 4), is assumed to lie in the cytoplasm.

Codon usage. Of the 61 codons, 2 (AGG and GAC) were not used in the *pgtP* gene. From the analysis of Grosjean and Fiers (8), the *pgtP* gene appears to preferentially use the degenerate codons found in the weakly expressed genes and has a codon preference statistic of 0.38, which was calculated as described by Sharp and Li (16). This suggests that the *pgtP* gene belongs to a group of genes with a low codon bias.

Amino acid sequence homology with components of hexose-6-phosphate and glycerol-3-phosphate transport systems. The PgtP polypeptide (406 amino acid residues) has a high degree of amino acid sequence similarity with the UhpT polypeptide (463 amino acid residues), the membrane-bound transporter for the hexose-6-phosphate transport system, which, like the *pgt* system, is expressed only in the presence of exogenous inducers (7, 19); with the UhpC polypeptide (219 amino acid residues), a membrane-bound regulatory protein that is presumably involved in inducer recognition and binding in the regulation of *uhpT* expression and which has a high degree of sequence homology with UhpT (7); and with the GlpT polypeptide (452 amino acid residues), a membrane-bound glycerol-3-phosphate transporter (5). Allowing for a few small gaps and a misalignment by one amino acid residue at the N terminus, the sequences of PgtP and UhpT aligned well, with identical amino acid residues occupying 31% of the positions (Fig. 5). Approximately the same degree of sequence similarity was observed between PgtP and UhpC polypeptides (comparison not shown). The sequences of PgtP and GlpT also aligned well, with identical amino acid residues occupying 37% of the positions. As expected from the pairwise similarity observed here and that between GlpT and UhpT reported previously (5, 7), a high degree of similarity was observed among GlpT, PgtP, and UhpT polypeptides; 17% of the 406 positions were occupied by identical amino acids (Fig. 5). In addition to the sequence similarities among GlpT, PgtP, and UhpT, the hydropathy profiles of these polypeptides were also extremely similar (data not shown). Eiglmeier et al. (5) have noted previously that the hydropathy profiles between GlpT and UhpT are similar. These observations reflect not only similar structures and functions among these polypeptides but also a common evolutionary origin for them.

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LITERATURE CITED

1. **Buchel, D. E., B. Gronenborn, and B. Muller-Hill.** 1980. Sequence of the lactose permease gene. Nature (London) **283**:541–545.
2. **Chang, A. C. Y., and S. N. Cohen.** 1978. Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid. J. Bacteriol. **134**: 1141–1148.
3. **Chen, L., D. Rhoad, and P. C. Tai.** 1985. Alkaline phosphatase and OmpA protein can be translocated posttranslationally into membrane vesicles of *Escherichia coli*. J. Bacteriol. **161**:973–980.
4. **Davis, R. W., D. Botstein, and J. Roth.** 1980. A manual for genetic engineering. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
5. **Eiglmeier, K., W. Boos, and S. T. Cole.** 1987. Nucleotide sequence and transcriptional startpoint of the *glpT* gene of *Escherichia coli*: extensive sequence homology of the glycerol-3-phosphate transport protein with components of the hexose-6-phosphate transport system. Mol. Microbiol. **1**:251–258.
6. **Engelman, D. M., T. A. Steitz, and A. Goldman.** 1986. Identifying nonpolar transbilayer helices in amino acid sequences of membrane proteins. Annu. Rev. Biophys. Chem. **15**:321–353.
7. **Friedrich, M. J., and R. J. Kadner.** 1987. Nucleotide sequence of the *uhp* region of *Escherichia coli*. J. Bacteriol. **169**:3556–3563.
8. **Grosjean, H., and W. Fiers.** 1982. Preferential codon usage in procaryotic genes: the optimal codon-anticodon interaction energy and the selective codon usage in efficiently expressed genes. Gene **18**:199–209.
9. **Henikoff, S.** 1984. Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. Gene **28**:351–359.
10. **Hu, M. C.-T., and N. Davidson.** 1986. Mapping transcription start point on cloned genomic DNA with T4 DNA polymerase: a precise and convenient technique. Gene **42**:21–29.
11. **Hugenholz, J., J.-S. Hong, and H. R. Kaback.** 1981. ATP-driven active transport in right-out membrane vesicles. Proc. Natl. Acad. Sci. USA **78**:3446–3449.
12. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
13. **Nohno, T., T. Saito, and J.-S. Hong.** 1986. Cloning and complete nucleotide sequence of the *Escherichia coli* glutamine permease operon (glnHPQ). Mol. Gen. Genet. **205**:260–269.
14. **Saier, M. H., Jr., D. L. Wentzel, B. U. Feucht, and J. J. Judice.** 1975. A transport system for phosphenolpyruvate, 2-phosphoglycerate, 3-phosphoglycerate in *Salmonella typhimurium*. J. Biol. Chem. **250**:5089–5096.
15. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
16. **Sharp, P. M., and W.-H. Li.** 1986. Codon usage in regulatory genes in *Escherichia coli* does not reflect seleltion for 'rare' codons. Nucleic Acids Res. **14**:7737–7749.
17. **Tabor, S., and C. C. Richardson.** 1985. A bacteriophage T7 RNA polymerase-promoter system for controlled exclusive expression of specific genes. Proc. Natl. Acad. Sci. USA **82**: 1074–1078.
18. **Vogel, H. J., and D. M. Bonner.** 1965. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**:97–102.
19. **Weston, L. A., and R. J. Kadner.** 1987. Identification of Uhp polypeptides and evidence for their role in exogenous induction of the sugar phosphate transport system of *Escherichia coli*. J. Bacteriol. **169**:3546–3555.
20. **Wilbur, W. J., and D. J. Lipman.** 1983. Rapid similarity searches of nucleic acid and protein data banks. Proc. Natl. Acad. Sci. USA **80**:726–730.
21. **Yu, G.-Q., and J.-S. Hong.** 1986. Identification and nucleotide sequence of the activator gene of the externally induced phosphoglycerate transport system of *Salmonella typhimurium*. Gene **45**:51–57.