Cloning, Characterization, and Nucleotide Sequence Analysis of a Zymomonas mobilis Phosphoglucose Isomerase Gene That Is Subject to Carbon Source-Dependent Regulation

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The Zymomonas mobilis gene encoding phosphoglucose isomerase (pgi) was cloned by genetic complementation of an Escherichia coli pgi mutant. An enzyme assay and sodium dodecyl sulfate-polyacrylamide gel electrophoresis confirmed the presence of excess amounts of phosphoglucose isomerase in E. coli clones carrying the Z. mobilis pgi gene. The pgi gene is present in only one copy on the Z. mobilis genome. Nucleotide sequence analysis of the pgi region revealed an open reading frame of 1,524 bp preceded by a strong Shine-Dalgarno sequence. The pgi gene encodes a 507-amino-acid protein with a predicted molecular weight of 55,398. Z. mobilis phosphoglucose isomerase is between 38 and 43% identical to the enzyme from other species. Northern (RNA) blot analysis showed that the pgi transcript is 1.8 kb in length. The level of the pgi transcript was found to be influenced by the phase of growth and by the carbon and energy sources. Transcript levels increased with respect to total RNA during logarithmic growth and were threefold higher when grown on fructose than on glucose. These changes in transcript levels paralleled phosphoglucose isomerase activities in the cultures. Differential mRNA stability was not a factor, since the half-life of the pgi transcript was 6.3 min in glucose-grown cells and 6.0 min in fructose-grown cells. Thus, an increase in the rate of transcription appears to be at least partially responsible for the increased levels of phosphoglucose isomerase observed for Z. mobilis grown on fructose.

Zymomonas mobilis has a simple, fermentative metabolism and lacks the complicated variety of metabolic alternatives encountered in other organisms (35). This microorganism is most commonly found in warm, tropical climates in fermentations of plant saps with high sugar concentrations. It uses the Entner-Doudoroff glycolytic pathway exclusively for catabolism of glucose, fructose, and sucrose, the only carbon and energy sources that support its growth. The Entner-Doudoroff pathway is inherently inefficient and yields only 1 mol of ATP per mol of glucose consumed. In order to obtain sufficient energy for growth, Z. mobilis must convert a significant amount of substrate to end products. This is facilitated by high level expression of glycolytic and alcoholic enzymes (approximately 50% of the total soluble protein). Z. mobilis is capable of near quantitative fermentation of glucose to equimolar amounts of ethanol and carbon dioxide with a specific ethanol productivity nearly threefold higher than that for Saccharomyces cerevisiae (26, 35).

Attempts to understand the regulation of carbon flow through metabolic pathways have led to the development of mathematical models of metabolic flux control which are based on enzyme kinetics (17, 20). Z. mobilis provides a useful experimental system for testing the validity of metabolic control theories. In this organism, several of the enzymes of the Entner-Doudoroff pathway are thought to operate at or near substrate saturation with no substantial allosteric control points (1). Therefore, many of the enzymes of the pathway contribute to overall metabolic flux control. It follows that Z. mobilis must possess a mechanism for carefully maintaining appropriate levels of certain enzymes of central metabolism. The concept of genetic regulation of constitutive enzymes is perhaps not widely appreciated, but it must certainly be relevant for expression of the glycolytic enzymes of Z. mobilis. It is our goal to analyze the genetic component of metabolic flux control in the Z. mobilis Entner-Doudoroff pathway. The first step in this investigation is to clone the genes that code for the enzymes of central metabolism. These cloned genes will be used in future studies as tools to manipulate the in vivo levels of specific glycolytic enzymes in order to assess their contribution to metabolic flux control.

Phosphoglucose isomerase is a dimeric enzyme with an average subunit size of 60 to 65 kDa (24). It catalyzes the reversible interconversion of glucose-6-phosphate to fructose-6-phosphate. In Z. mobilis, phosphoglucose isomerase serves as part of a peripheral pathway which makes it possible for the organism to utilize fructose as a substrate for growth (35). Fructose is taken up by facilitated diffusion and is phosphorylated by fructokinase to form fructose-6-phosphate. Phosphoglucose isomerase then converts fructose-6-phosphate to glucose-6-phosphate, an intermediate of the Entner-Doudoroff pathway. The role of phosphoglucose isomerase in this peripheral route for fructose metabolism is quite different from its role as a central step in the Embden-Meyerhof-Parnas pathway, where it serves to isomerize glucose-6-phosphate to fructose-6-phosphate, which is phosphorylated by phosphofructokinase to form fructose-1,6-biphosphate, the key intermediate of this pathway.

Phosphoglucose isomerase and fructokinase activities in Z. mobilis cells are adequate to support the flux observed when Z. mobilis is grown on fructose (1, 26, 35). When Z.

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**TABLE 1. Plasmid and strains used in this study**

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<th>Strain or plasmid</th>
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<td><em>E. coli</em> DH5α</td>
<td>lacZΔM15 recA</td>
<td>BRL*</td>
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<td>DF214</td>
<td>Δ(zwf- Eddie) pgi::Mu</td>
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<td><strong>Plasmids</strong></td>
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</tr>
<tr>
<td>pTC132</td>
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</table>

* Bethesda Research Laboratories.

*b* Incomplete lacI and lacZ genes.

*Z. mobilis* is grown on glucose, the levels of these two enzymes are lower than those required for growth on fructose. Fructokinase activity has been shown to increase when cells are grown on fructose (12, 29). Data obtained in this laboratory show the same to be true for phosphoglucone isomerase. In this paper the cloning, characterization, expression, and sequence analysis of the phosphoglucone isomerase gene from *Z. mobilis* are described.

**MATERIALS AND METHODS**

**Bacterial strains.** The bacterial strains and plasmids used for this study are listed in Table 1. *Zymomonas mobilis* CP4 was the source of the DNA used to construct the genomic library from which phosphoglucone isomerase was cloned. This strain was grown at 30°C in yeast extract-peptone (YPE) medium (25) containing 100 g of carbohydrate per liter. *Escherichia coli* DF214, which bears a Mu phage insertion in pgi as well as a deletion of the zwf- Eddie linkage group, was used for cloning pgi by complementation (36). *E. coli* DH5α was used as a utility strain for DNA amplification. *E. coli* strains were maintained in Luria broth (21). Solid media were prepared with 1.5% agar. Complementation of *E. coli* DF214 was accomplished on minimal medium (34) with the appropriate antibiotic and with glucose as the sole carbon and energy source. *E. coli* transformants containing pUC18 derivatives were selected on LB plates supplemented with X-Gal (5-bromo-4-chloro-3-indoly-β-D-galactopyranoside) (20 mg/liter) and 50 μg of ampicillin per ml.

**Preparation and manipulation of plasmid and genomic DNA.** Procedures for transformation and restriction mapping followed standard methods (27, 30). Small-scale plasmid preparations were carried out by using a modification of the method of Birnboim and Doly as described previously (30). Restriction enzymes and DNA modification enzymes were used according to the recommendations of the manufacturers. Genomic DNA from *Z. mobilis* CP4 was isolated as previously described (3). A library of *Z. mobilis* genomic DNA containing 4- to 6-kb fragments was generated by partial digestion of genomic DNA with *Sau3A* and subsequent ligation into the BamHI site of pUC18 as described previously (2). Amplification in *E. coli* DH5α produced a 10,000-member library with a 75% insertion frequency.

**Cloning of the *Z. mobilis* gene encoding phosphoglucone isomerase.** The *Z. mobilis*-pUC18 library was transformed into *E. coli* DF214 [pgi::Mu Δ(zwf- Eddie)] (36)]. Clones were selected for restored ability to grow on glucose minimal medium. Confirmation of phosphoglucone isomerase clones was made by high frequency transformation of *E. coli* DF214 to the glucose + phenotype and by enzyme assay. The two *Z. mobilis* phosphoglucone isomerase clones selected for further study were designated pTC131 and pTC132.

**Enzyme assay of phosphoglucone isomerase.** *Z. mobilis* CP4, *E. coli* DH5α(pTC131), and *E. coli* DH5α(pTC132) were prepared for enzyme assay by washing in 50 mM potassium phosphate buffer (pH 6.8) and then resuspending in the same buffer to an *A* max of 1.0. Two milliliters of cell suspension was pelleted and resuspended in 500 μl of phosphate buffer. *Z. mobilis* cell lysates (500 μl) were prepared by sonication with three 15-s bursts in a microcentrifuge tube containing one-half volume of 0.1-mm-diameter glass beads. *E. coli* cell lysates were prepared by sonication for 15 s. Phosphoglucone isomerase activity was assayed as described previously (18).

**Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis.** Gels were prepared and stained with Coomassie blue as previously described (8).

**Southern probing.** Electrophoresis and ethidium bromide staining of agarose gels (0.8%) for Southern blotting were performed by standard methods (27), as described previously (7). Nucleic acid transfer to GeneScreen Plus hybridization transfer membranes and hybridization of labeled probes at 42°C in the presence of 50% formamide were performed according to the manufacturer’s protocols (New England Nuclear Corp., Boston, Mass.; catalog no. NEF-976). Random-primed DNA labeling was used to prepare the hybridization probes.

**Analysis of RNA.** RNA was isolated by a modification of the procedure described by Mackie (22). Log-phase *Z. mobilis* cultures (5 ml) were pipetted into 2.5 ml of a mixture containing 0.3 M sodium acetate (pH 6.0), 30 mM EDTA, and 3% SDS at 100°C in a boiling water bath. Complete cell lysis was accomplished within 30 s. This was followed by two successive extractions with phenol at 60°C and a single extraction with chloroform at room temperature. These samples were precipitated with ethanol, and the DNA was removed as described previously (8). Northern (RNA) analysis was carried out as described previously (7). For mRNA half-life determinations, exponential cultures were treated with rifampin (200 mg/liter) as described previously (22). RNA samples were taken at 3-min intervals thereafter and quantitated before loading equal amounts on denaturing agarose gels for Northern analysis as described above. The amount of radioactive probe that hybridized to the filters was quantitated by using the AMBIS Radioanalytic Imaging System (AMBIS Systems, Inc., San Diego, Calif.).

**Determination of DNA sequence.** The DNA sequences of the *Z. mobilis* pgi regions on pTC131 and pTC132 were determined using the dyeoxy method (28). Double-stranded sequencing was performed with the aid of a Sequenase kit as described (5). A series of deletion subclones (average length, 300 bp) for pTC131 and pTC132 was generated with exonuclease III (Erase-a-Base kit). Sequence analysis of both strands required the use of 47 deletion subclones which composed a single contiguous sequence of 3,212 bp encompassing the *Z. mobilis* phosphoglucone isomerase gene and relevant 5’ and 3’ regions. Sequence data were compiled with the University of Wisconsin Genetics Computer Group Sequence Analysis Software Package version 6.1 (11).

**Enzymes and chemicals.** Restriction enzymes were obtained from Bethesda Research Laboratories (Gaithersburg, Md.) and from Promega Corporation (Madison, Wis.). Sequenase and random-primed DNA labeling kits were purchased from U.S. Biochemical Corp. (Cleveland, Ohio). The Erase-a-Base system used for generating deletion clones was...
purchased from the Promega Corp. Radioactive compounds were ordered from New England Nuclear Corp. Sigma Chemical Co. (St. Louis, Mo.) was the source of the biochemicals used in this study.

**Nucleotide sequence accession number.** The entire nucleotide sequence shown in Fig. 4 is available on the GenBank data base, accession no. M62957.

**RESULTS**

Cloning and characterization of the gene encoding phosphoglucose isomerase. The cloning of phosphoglucose isomerase from *Z. mobilis* was accomplished by genetic complementation of an *E. coli* mutant. Mutations in the phosphoglucose isomerase and glucose-6-phosphate dehydrogenase genes in *E. coli* DF214 prevent growth of the organism on glucose minimal medium. Theoretically, complementation of either of these mutations with *Z. mobilis* genes would lead to restored ability to grow on glucose. *E. coli* DF214 was transformed with the *Z. mobilis* genomic library and plated on minimal glucose medium containing ampicillin. Restoration of the ability to grow on glucose was observed in approximately 1 in 500 transformants. Of the over 150 glucose+ clones which originally appeared, 35 were selected for further study. High-frequency transformation of *E. coli* DF214 to the glucose+ phenotype was observed for plasmid DNA prepared from each of these 35 clones. Enzyme assays were performed to distinguish between clones expressing phosphoglucose isomerase and those expressing glucose-6-phosphate dehydrogenase. Of the 35 original clones, 34 were found to express phosphoglucose isomerase. The levels of phosphoglucose isomerase expression by these clones, on the average, were between 10-fold (2.3 μmol/min/mg of total cell protein) and 25-fold (5.8 μmol/min/mg of total cell protein) higher than the wild-type levels present in *E. coli* DH5α. Two of these clones were chosen for further study (pTC131 and pTC132). The remaining glucose+ clone expressed high glucose-6-phosphate dehydrogenase activity. A study of the *Z. mobilis* zwf gene was published elsewhere (2).

Restriction maps of pTC131 and pTC132 were constructed (Fig. 1). Nested deletions were generated with exonuclease III by opening the clones on the 5’ end with *SmaI* and by blocking with *SstI*. Deletions made from the 3’ end were opened with *XbaI* and blocked with *PstI*. The *pgi* reading frame was localized by using an enzyme assay of the deletion subclones to determine which deletions eliminated necessary portions of the gene. The *pgi* genes in both pTC131 and pTC132 were found to be in the same orientation (with respect to direction of transcription) as the pUC18-borne lac promoter.

SDS-polyacrylamide gels of extracts of *E. coli* DH5α (pTC131) and *E. coli* DH5α (pTC132) revealed a prominent polypeptide with an apparent mobility of approximately 50,000 Da (Fig. 2). Enzyme assays confirmed that these clones overexpressed phosphoglucose isomerase activity. Deletions of pTC131 or pTC132 that eliminated phosphoglucose isomerase activity resulted in the loss of this polypeptide from SDS-polyacrylamide gels. For example, the deletion subclone pTC131.1C (in *E. coli* DF214) lacked

![FIG. 1. Restriction maps of the *pgi* region and the original *pgi* clones from the *Z. mobilis* library. The region sequenced (3,212 bp) is shown on the top line. A 0.5-kb size marker is provided. The orientation of the *lac* promoter (pUC18) in clones pTC131 and pTC132 is shown. Only the *Z. mobilis* DNA inserted into the vector is shown. Restriction sites are designated as follows: S, *SmaI*; RV, *EcoRI*; N, *NruI*; Nt, *NotI*; M, *MluI*; K, *KpnI*; B, *BamHI*; Nd, *NdeI*. The asterisk indicates that there is more than one restriction site within 15 bp.](http://jb.asm.org/)
Expression of *pgi*. Phosphoglucone isomerase activity was twofold higher for early-log-phase cultures of *Z. mobilis* grown on fructose than for those grown on glucose. Phosphoglucone isomerase activity in early-log-phase cultures of fructose-grown cells was 1.32 μmol/min/mg of protein, while that of glucose-grown cells was 0.65 μmol/min/mg of protein. As the cultures reached late log phase, the fructose-grown cells contained activity nearly threefold higher than that of glucose-grown cells. For late-log-phase cultures, the phosphoglucone isomerase activity of fructose-grown cells was 2.78 μmol/min/mg of protein, while that of glucose-grown cells was 1.04 μmol/min/mg of protein. These data also indicate that phosphoglucone isomerase levels increased approximately twofold during logarithmic growth on either glucose or fructose, as has been observed for several other glycolytic enzymes of *Z. mobilis* (25).

Northern blot analysis revealed that the gene encoding *pgi* resides on a 1.8-kb transcript (Fig. 5). This transcript is longer than the 1.524 bases needed to encode the structural gene. Although long 5' untranslated regions of 200 bases or more are not uncommon in *Z. mobilis* (6, 9, 10, 13), primer extension data indicated that *pgi* transcription is initiated 75 bases upstream of the start codon (data not shown). The nucleotide sequence upstream of the putative transcriptional start site does not show any significant matches to the proposed consensus promoter sequence for *Z. mobilis* (19). The T-rich stem-loop structure referred to above lies approximately 200 bases downstream of the *pgi* stop codon and may delineate the end of the 1.8-kb transcript, which would include the 75-base 5' untranslated region, the 1.524-base reading frame, and the 200-base 3' untranslated region.

Northern analysis of *pgi* mRNA levels in *Z. mobilis* cells grown both on fructose and on glucose was performed in order to determine whether the elevation in enzyme activity was the result of transcriptional regulation (Fig. 5). The levels of *pgi* mRNA in cells grown on fructose were found to be approximately threefold higher than the levels observed in cells grown on glucose. This threefold increase was seen for cells in early log phase as well as late log phase. In addition, the *pgi* message appeared to accumulate during logarithmic growth. Cells harvested in late log phase from either glucose or fructose medium contained *pgi* transcript levels approximately threefold higher than those of early-log-phase cells grown in the same medium. It was of interest to determine whether the increased message levels observed for fructose cultures were a general phenomenon for the genes that encode glycolytic enzymes in *Z. mobilis* or whether they were restricted to *pgi*. Filters prepared with the same RNA samples were subjected to hybridization with gene-specific probes for *gap* (coding for glyceraldehyde phosphate dehydrogenase [9]), *pgk* (coding for phosphoglycerate kinase [6]), *pdc* (coding for pyruvate decarboxylase [8]), and *adhB* (coding for alcohol dehydrogenase II [10]). These experiments showed that equivalent amounts of the specific *gap*, *pgk*, *pdc*, and *adhB* mRNAs were present in both glucose and fructose cultures (data not shown).

In order to determine whether the increased levels of the *pgi* transcript in fructose cultures were the result of differential transcript stability, the half-life of the *pgi* mRNA was determined in glucose and fructose cultures. The results of these experiments for the two cultures are shown in Fig. 5. Decay of the *pgi* transcript in both cultures followed a similar pattern and was biphasic. The decay rates doubled when approximately 20% of the transcript remained. The measured half-life (during the initial phase of decay) of the *pgi* transcript in glucose-grown cells was 6.3 ± 0.4 min. The

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**Fig. 3.** Southern blot analysis of genomic DNA from *Z. mobilis* and *E. coli* hybridized with a *pgi* gene-specific probe. Size markers are provided (in kilobases) on the right. *E. coli* DNA was loaded in lane 1, and *Z. mobilis* DNA was loaded in lanes 2 through 7. DNA was digested with EcoRI (lanes 1 and 2), EcoRV (lane 3), HindIII (lane 4), KpnI (lane 5), PstI (lane 6), and Sall (lane 7). Phosphoglucone isomerase activity, and protein extracts from this clone (in *E. coli* DH5α) lacked the prominent polypeptide on SDS-polyacrylamide gels that was associated with high levels of phosphoglucone isomerase activity in positive clones (Fig. 2). The reading frame was localized in this fashion to within roughly 300 bp.

Origination of the clones from *Z. mobilis* was confirmed by Southern blot analysis (Fig. 3). A gene-specific probe was generated by digesting pTC131 with BamHI and KpnI, producing a probe of 174 bases in length. Hybridization to specific restriction fragments of *Z. mobilis* genomic DNA was observed. No hybridization to *E. coli* W3110 genomic DNA (digested with EcoRI) could be detected. The hybridization pattern observed with *Z. mobilis* genomic DNA indicated that *pgi* exists as a single-copy gene on the genome.

**Nucleotide sequence analysis of phosphoglucone isomerase.** The sequence of the gene that specifies phosphoglucone isomerase in *Z. mobilis* is presented in Fig. 4. The *pgi* gene consists of an open reading frame of 1,524 bp, corresponding to a 507-amino-acid protein with a predicted molecular weight of 55,398. The *pgi* reading frame is preceded by a Shine-Dalgarno sequence 9 bases upstream of the ATG initiation codon (31). The *pgi* gene stops with the codon TAA. Computer analysis did not predict a strong terminator downstream of the gene (GGC, TERMINATOR program). However, a prominent stem-loop structure (12 paired bases with a loop of 7 bases) was found beginning at base 2592. In addition, a set of three partially overlapping stem-loop structures was identified beginning at base 2686 and ending at base 2750. This region includes numerous thymidines and might act as a transcriptional terminator.
<table>
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FIG. 4. Nucleotide sequence of the Z. mobilis pgil gene. The reading frame begins with a Met start codon. The Shine-Dalgarno sequence is underlined. The stop codon is designated by End. Numbering begins at the first base sequenced.
FIG. 5. Northern blot analysis of the _Z. mobilis_ pgi transcript with a gene-specific hybridization probe. (A) Lanes containing RNA samples from cells grown under different conditions are as follows: 1, late-log-phase fructose-grown culture; 2, early-log-phase fructose-grown culture; 3, late-log-phase glucose-grown culture; 4, early-log-phase glucose-grown culture. RNA (10 µg) was loaded in each lane. Size markers (in kilobases) are provided on the right. (B and C) Transcript stability in a glucose-grown (B) and in a fructose-grown (C) culture. Total RNA was harvested from log-phase cultures after inhibition of transcription at the indicated times (in minutes). Gels were loaded with 2.5 µg of RNA per lane. Size markers (in kilobases) are provided on the left. (D) A semilogarithmic plot of the data shown in panels B and C, quantitated by scanning of the filters with the AMBIS system. Decay rate of the pgi mRNA in glucose-grown cells (○) was 6.3 ± 0.4 min; decay rate in fructose-grown cells (X) was 6.0 ± 0.8 min. Decay rates were determined from the initial rates of the biphasic curves.

**Quence of the** _pgi_ **coding region, with the amino acid sequence of** _E. coli_ **phosphogluco isomerase is shown in** Fig. 6. **Phosphogluco isomerase from** _Z. mobilis_ **exhibited 43% sequence identity to the** _E. coli_ **phosphogluco isomerase** (14). **The results of comparisons of the amino acid sequence of the** _Z. mobilis_ **pgi product with the amino acid sequences of phosphogluco isomerase enzymes from** _Kluyveromyces lactis_ (37), _Clarkia unguiculata_ (32), _Trypanosoma brucei_ (23), _S. cerevisiae_ (33), and pigs (4) are shown in Table 2. On the average, phosphogluco isomerase from _Z. mobilis_ is 40% identical to the eukaryotic enzymes. In addition, the _Z. mobilis_ enzyme is 38% identical to mouse neuroleukin, an enzyme that is 90% identical to phosphohexose isomerase (16). Comparison with a eukaryotic active site peptide did not reveal substantial identity (15).

**DISCUSSION**

The _Z. mobilis_ gene encoding phosphogluco isomerase was cloned by genetic complementation of _E. coli_ DF214. Biochemical assays of the enzymes produced by _E. coli_ DF214(pTC131) and _E. coli_ DF214(pTC132) confirmed the identity of these phosphogluco isomerase clones. SDS-polyacrylamide gel electrophoresis of _E. coli_ DH5α(pTC131) and _E. coli_ DH5α(pTC132) extracts revealed an overexpressed protein of approximately 50,000 Da. Deletion subclones generated from pTC131 and pTC132 that extended
into the structural gene resulted in loss of phosphoglucose isomerase activity and absence of the protein on the polyacrylamide gel.

Southern blot analysis showed that the pgi gene is present in a single copy on the Z. mobilis genome. All the genes encoding glycolytic enzymes from Z. mobilis studied thus far are present in single copies (2, 19). The pgi open reading frame begins with an AUG start codon that is preceded 9 bp upstream by a strong Shine-Dalgarino sequence (31). The strong ribosome-binding site would allow efficient translation of the pgi message, as is also the case for the other genes that encode glycolytic enzymes of Z. mobilis (2, 19). Primer extension data indicated a start site 75 bp upstream of the start codon, but the nucleotide sequence upstream of the putative transcriptional start site does not resemble the consensus promoter sequence proposed for Z. mobilis (19). A more extensive examination of the pgi promoter is indicated. No strong terminator was predicted from the sequence downstream of the UAA stop codon. However, a region of prominent stem-loop structures approximately 200 bp downstream of the stop codon might serve to terminate transcription.

Northern blot analysis revealed that pgi is encoded on a 1.8-kb transcript. The level of the pgi transcript was approximately threefold higher in late-log-phase cells than in early-log-phase cultures grown on either glucose or fructose. Phosphoglucose isomerase activity also showed a comparable increase during logarithmic growth. Similar results have been obtained for most of the glycolytic enzymes from Z. mobilis (25). These results suggest that the glycolytic enzymes are synthesized at a faster rate than the majority of proteins in Z. mobilis. Likewise, the accumulation of the pgi transcript during logarithmic growth might indicate that the rate of pgi transcription is faster than that of the bulk of unstable mRNA or alternatively, that its rate of decay is slower than that of the bulk of unstable RNA. Measurements of mRNA half-life indicate that the pgi transcript has a decay rate of approximately 6.3 min in glucose-grown cells and 6.0 min in fructose-grown cells. However, at this stage we do not know the average stability of Z. mobilis mRNA. We are currently addressing this question.

Even more interesting is the finding that pgi transcript levels in fructose-grown cells are approximately threefold higher than message levels in glucose-grown cells. This parallels the increase in phosphoglucose isomerase Activity in cells grown on fructose. Similar experiments with four gene-specific probes for other steps of central metabolism showed that these were not subject to carbon source-dependent regulation, indicating that this is not a general phenomenon. The data presented in this paper suggest that an increase in the rate of pgi transcription during growth on fructose, as opposed to increased transcript stability (slower decay rate), is at least partially responsible for the increase in phosphoglucose isomerase synthesis observed in fructose cultures.

Z. mobilis has become highly specialized during the course of its evolution for growth in a high sugar habitat and is the only bacterium that uses the Entner-Doudoroff pathway anaerobically. This is remarkable, considering the inherent inefficiency of this pathway for energy metabolism. The rapid carbon flux that is necessitated by inefficient energy production is facilitated by high levels of the pathway enzymes, which are adequate to support the metabolic fluxes observed (1, 35). Phosphoglucose isomerase plays a dual role in Z. mobilis. It is an essential component of the peripheral pathway that brings fructose into central metabolism. It is also employed for production of fructose-6-phosphate, a metabolic precursor for bio synthesis, from glucose-6-phosphate during growth on glucose. The level of phosphoglucose isomerase in early-log-phase glucose cultures is not sufficient to allow the flux observed when it is grown on fructose (1, 26). This presumably is the reason that phosphoglucose isomerase levels are increased in Z. mobilis.

**TABLE 2. Phosphoglucose isomerase amino acid comparisons**

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a Amino acid sequences of phosphoglucose isomerases of the organisms shown were compared with that of Z. mobilis.
when it is grown on fructose. The evolution of a mechanism for carbon source-dependent regulation of a constitutive enzyme is extremely interesting. We are currently investigating the molecular aspects of this regulation.

Comparison of the primary amino acid sequence deduced from the Z. mobilis pgI gene with phosphoglucone isomerase sequences from other organisms revealed higher identity to the prokaryotic sequence from E. coli (43%) than to the eukaryotic organisms (40% average identity). It is perhaps somewhat surprising that Z. mobilis phosphoglucone isomerase is not more highly conserved, since it would be very beneficial to an organism like Z. mobilis, which is forced to use most of its available substrate for energy production, to have the most efficient enzymes possible. On the other hand, the amino acids which are conserved between Z. mobilis phosphoglucone isomerase and the other enzymes are likely to be those that are most critical for enzyme function. Further comparisons of enzyme structures will give a better picture of the evolutionary relationships between glycolytic enzymes in Z. mobilis and those in other species.

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