JOURNAL OF BACTERIOLOGY, June 1992, p. 3455–3460
0021-9193/92/113455-06$02.00/0

Vol. 174, No. 11

Cloning, Sequencing, and Expression of the *Zymomonas mobilis* Fructokinase Gene and Structural Comparison of the Enzyme with Other Hexose Kinases

BOZENA ZEMBRZUSKI,[1] PETER CHILCO,[1] XIAO-LI LIU,[1] JIAN LIU,[2] TYRRELL CONWAY,[2] AND ROBERT SCOPES[1]*

Centre for Protein and Enzyme Technology and Department of Biochemistry, La Trobe University, Bundoora, Victoria 3083, Australia,[1] and School of Biological Sciences, University of Nebraska, Lincoln, Nebraska 68588-0118[2]

Received 30 September 1991/Accepted 24 March 1992

The *frk* gene encoding the enzyme fructokinase (fructose 6-phosphotransferase [EC 2.7.1.4]) from *Zymomonas mobilis* has been isolated on a partial *Taq*I digest fragment of the genome and sequenced. An open reading frame of 906 bp corresponding to 302 amino acids was identified on a 3-kbp *Taq*I fragment. The deduced amino acid sequence corresponds to the first 20 amino acids (including an N-terminal methionine) determined by amino acid sequencing of the purified protein. The 118 bp preceding the methionine codon on this fragment does not appear to contain a promoter sequence. There was weak expression of the active enzyme in the recombinant *Escherichia coli* clone under control of the *lac* promoter on the pUC plasmid. Comparison of the amino acid sequence with that of the glucokinase enzyme (EC 2.7.1.2) from *Z. mobilis* reveals relatively little homology, despite the fact that fructokinase also binds glucose and has kinetic and structural properties similar to those of glucokinase. Also, there is little homology with hexose kinases that have been sequenced from other organisms. Northern (RNA) blot analysis showed that the *frk* transcript is 1.2 kb long. Fructokinase activity is elevated up to twofold when *Z. mobilis* was grown on fructose instead of glucose, and there was a parallel increase in *frk* mRNA levels. Differential mRNA stability was not a factor, since the half-lives of the *frk* transcript were 6.2 min for glucose-grown cells and 6.6 min for fructose-grown cells.

Zymomonas mobilis is capable of growing on and fermenting only glucose, fructose, and sucrose (29, 30). It has been reported by several workers that approximately half of the sucrase activity of *Z. mobilis* cells is excreted into the medium (20, 21); the remainder appears to be in the periplasmic space, with possibly some in the cell (14, 22). Thus, sucrose is mostly hydrolyzed before entering the cell. The monomer sugars glucose and fructose are transported by facilitated diffusion into the cell (9), where they are acted on by specific kinases which phosphorylate them in position 6 (10, 11, 27). Fructose 6-phosphate is converted by phosphoglucose isomerase to glucose 6-phosphate, which then enters the central Entner-Doudoroff glycolytic pathway. Thus, fructokinase and phosphoglucose isomerase constitute a pathway that allows *Z. mobilis* to utilize fructose as an energy source. When grown on glucose, neither of these enzymes is present at levels that would be sufficient to maintain the fermentation flux if the sugar substrate were suddenly switched to fructose (1), although the shortfall is less than a factor of 2. Fructokinase activity increases by approximately twofold when the cells are maintained on fructose, instead of glucose (33). A nearly fourfold increase was reported by Doelle (10), but in this case the growth conditions were not comparable. Phosphoglucose isomerase activity also increases between two- and threefold (15) when cells are grown on glucose. It was shown that the elevation in phosphoglucose isomerase activity was the result of increased transcription (15). Thus, it was of interest to see whether the mechanism of control of fructokinase activity was similar.

Both glucokinase and fructokinase of *Z. mobilis* have been purified and characterized (27); each is a dimeric protein, with subunit sizes estimated from sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis of 33 and 30 kDa, respectively. Kinetic properties are similar, with high specificity for each substrate; however, fructokinase is strongly competitively inhibited by glucose, but glucokinase is not inhibited by fructose. In view of the similarity of the two enzymes, structural studies have been undertaken to compare them. The genes encoding both enzymes have been isolated in our laboratories using enzyme activity staining of clonal libraries. The gene for glucokinase has been isolated and sequenced as part of an operon including two other glycolytic enzymes and a putative glucose transporter protein (4); the present article presents the sequence of the fructokinase gene, compares the deduced sequence of the structural protein with the sequences of similar hexose kinase enzymes and of *Z. mobilis* glucokinase, and demonstrates that the increased expression in fructose-grown cells is due to increased transcription of the gene.

MATERIALS AND METHODS

Bacterial strains. *Z. mobilis* ZM6 (ATCC 29191) was obtained from P. L. Rogers, University of New South Wales, Sydney, Australia. Recombinant DNA libraries of *Escherichia coli* JM101 were constructed with pUC9 vectors as described previously (21) by using *Sau*3A partial digest fragments cloned into the *Bam*HI site and partial *Taq*I digests cloned into the phosphatase-treated *Acc*I site.

Screening of libraries for expression of fructokinase. Colonies were transferred to and lysed on nitrocellulose paper. Enzymic activity was detected on the nitrocellulose paper by

* Corresponding author.

TABLE 1. Fructokinase specific activity in extracts of *Z. mobilis* cells grown on different sugars and in extracts of fructokinase-positive *E. coli* clones

Cells	Fructokinase sp. act (U/mg of protein)
Z. mobilis grown on:	
Glucose (15%)	1.9
Fructose (15%)	3.6
Sucrose (15%)	3.0
E. coli containing plasmid:	
pUC9	<0.05
pZFK1	1.5
pZFK2	1.6
pZFK1 (glucose suppressed)	0.4

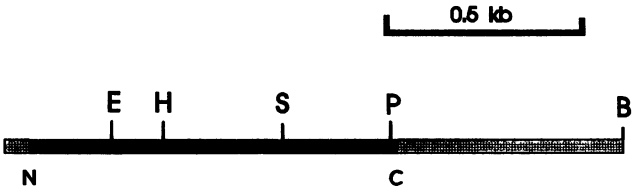

FIG. 1. Restriction map of the insert in plasmid pZFK2. The solid area represents the region encoding the fructokinase protein (N-terminal end [N] to C-terminal end [C]). Restriction sites utilized in subcloning: E, *Eco*RI; H, *Hin*dIII; P; *Pst*I; B, *Bam*HI; S, *Sau*3A.

fluorescence of NADH using a mixture of 10 mM fructose, 5 mM ATP, 10 mM $MgCl_2$, 2 mM NAD^+, 5 U of *Z. mobilis* glucose 6-phosphate dehydrogenase per ml (27), and 5 U of rabbit muscle phosphoglucose isomerase per ml (26) in 20 mM phosphate buffer (pH 7). A piece of Whatman No. 1 filter paper was soaked in the detection mixture and placed on nitrocellulose paper containing lysed colonies. After 5 to 30 min, positive clones could be seen under near-UV light by the blue-green fluorescence of NADH produced at the sites of enzyme activity.

DNA sequence analysis. Restriction fragments of the genes were subcloned into vector pUC18 or pUC19 and sequenced either by the double-stranded sequencing method (7) or by sequencing in M13 using *Taq* polymerase (Promega Inc.) (16).

Amino acid sequencing. Enzyme isolated from *Z. mobilis* ATCC 29191 as described previously (27) was subjected to amino acid sequencing on an Applied Biosystems model 470A gas-liquid-phase sequencer.

Analysis of RNA. Total RNA was isolated, and Northern (RNA) blot analysis of mRNA was carried out as described previously (15). The DNA hybridization probe used in this study was a 1.0-kb fragment extending from the 5′ cloning site to the *Pst*I site adjacent to the stop codon (Fig. 1 and 2) and was labelled using a random primed labelling kit (Promega Inc.) as described by the manufacturer. For mRNA half-life determinations, exponential-phase cultures were treated with rifampin (200 mg/liter) as described previously (15). 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.).

RESULTS

Cloning and sequencing of the gene for fructokinase (*frk*). The clone used for studying the fructokinase gene was found in the *Taq*I library and had an inserted DNA fragment only 3 kbp long. The plasmid in this recombinant clone was named pZFK1. The fructokinase specific activity was about the same as in glucose-grown *Z. mobilis* cells (Table 1). Subcloning using *Bam*HI enabled removal of approximately half of the 3′ end, to give a 1.5-kb fragment still expressing the enzyme (plasmid pZFK2). This was further digested with *Hin*dIII and *Pst*I into three nearly equal parts for convenient sequencing (Fig. 1).

The 1,080-bp sequence, including an open reading frame of 906 bp corresponding to the structural part of the fructokinase gene is shown in Fig. 2. N-terminal amino acid sequencing of the first 20 amino acids (shown in italics) of the enzyme was in agreement with the deduced sequence (Cys at position 10 was not identified in the amino acid sequencing) and commenced with the initiator methionine. Thus, the initiator methionine remains on the mature protein. The same applies to glucokinase; an amino acid sequence analysis of the first 40 amino acids of the N-terminal region of glucokinase agreed exactly with the deduced sequence (4), with methionine as the first amino acid. A possible ribosome-binding site GAGA in the *frk* gene is unusually close (3 bases) to the start codon; it is more likely that the true site resides in the GAGTGG sequence spaced 9 bases from the start codon, although the presence of a thymidine in this sequence is not matched by any other published *Z. mobilis* gene.

The isolated *Taq*I fragment commenced only 118 bp 5′ to the start codon, and it is likely that the natural promoter lies upstream from this. The sequence ends with tandem TAA stops, immediately followed by a palindromic sequence (15 matches of 20, underlined) indicated by arrowed lines that could serve as a transcriptional terminator (31). The molecular weight of the protein as deduced from the base sequence is 32,572.

Sequence comparison with *Z. mobilis* glucokinase and other hexose kinases. Although the macrostructures and kinetic properties of *Z. mobilis* glucokinase and fructokinase are very similar, the levels of homology for sequences are small. Glucokinase is 26 amino acids longer; similarities at the C terminus suggest that glucokinase extends for an extra 5 amino acids at this end. Comparison of the glucokinase sequence with *Saccharomyces cerevisiae* hexokinase (2, 17, 28) indicates that a putative ATP-binding site commences close to the N terminus and that a similar but smaller homology exists with fructokinase, which appears to have a 5-amino-acid addition at this end. Using the University of Wisconsin Genetics Computer Group Sequence Analysis program gave only low degrees of similarity between the sequences. To illustrate how different these two sequences are, an alignment which optimizes matches of clusters of mostly glycine and hydrophobic residues, with a minimum of gaps in the two sequences, is shown in Fig. 3.

Expression of *frk*. It has been reported that the expression level of fructokinase is some twofold greater in *Z. mobilis* cells grown on fructose than in glucose-grown cells (10, 33). This also applies when mixtures of glucose and fructose (33) and sucrose is used as the sugar (Table 1). The specific activities of enzyme from cells grown to stationary phase on between 10 and 15% sugar have been in the range of 1.4 to 1.9 U/mg of soluble protein for glucose, 2.5 to 3.6 U/mg for fructose, and intermediate values for glucose-fructose mixtures and for sucrose. Similar results and absolute numbers have been found for the other enzyme necessary for fructose metabolism, phosphoglucose isomerase (15).

Northern blot analysis showed that the *frk* gene resides on

```
                                                                    TCGATTTATTCA

  13  AAAGGCCTTTTGGAGAGAACAAAAATCGAGGTCATCGTCATAATTTAAAGCGAATGGACAGCATATACCTCCGTATTAC

  92  GGGGGGATTTTGTGAGTGGTGAGAATA ATG AAA AAC GAT AAA AAA ATT TAT GGA TGC ATT GAA GGC
                                  Met Lys Asn Asp Lys Lys Ile Tyr Gly Cys Ile Glu Gly

 158  GGC GGA ACG AAA TTT ATG CTC GCC CTT ATT GAT TCT GAC CGG AAG ATG CTG GCT GTG GAA
      Gly Gly Thr Lys Phe Met Leu Ala Leu Ile Asp Ser Asp Arg Lys Met Leu Ala Val Glu

 218  CGT GTT CCG ACC ACA ACC CCT GAA GAA ACG CTT GGT AAA AGT GTT GAA TTC TTC AAA AAA
      Arg Val Pro Thr Thr Thr Pro Glu Glu Thr Leu Gly Lys Ser Val Glu Phe Phe Lys Lys

 278  GCG CTT CCT CAA TAC GCT GAT TCT TTT GCG TCT TTT GGT ATC GCG TCA TTT GGC CCG CTT
      Ala Leu Pro Gln Tyr Ala Asp Ser Phe Ala Ser Phe Gly Ile Ala Ser Phe Gly Pro Leu

 338  TGC CTC GAT CGC AAA AGC CCG AAA TGG GGC TAT ATC ACC AAC ACC CCG AAA CCT TTC TGG
      Cys Leu Asp Arg Lys Ser Pro Lys Trp Gly Tyr Ile Thr Asn Thr Pro Lys Pro Phe Trp

 398  CCG AAT ACG GAC GTT GTA ACG CCC TTT AAA GAA GCT TTT GGT TGC CCG GTT GAA ATC GAC
      Pro Asn Thr Asp Val Val Thr Pro Phe Lys Glu Ala Phe Gly Cys Pro Val Glu Ile Asp

 458  ACC GAT GTG AAT GGC GCA GCT TTG GCT GAA AAC TTC TGG GGC GCA TCA AAA GGC ACC CAT
      Thr Asp Val Asn Gly Ala Ala Leu Ala Glu Asn Phe Trp Gly Ala Ser Lys Gly Thr His

 518  ACA TCT GTT TAT GTC ACG GTT GGC ACC GGC TTC GGC GGT GGC GTA CTG ATT GAT GGT AAA
      Thr Ser Val Tyr Val Thr Val Gly Thr Gly Phe Gly Gly Gly Val Leu Ile Asp Gly Lys

 578  CCG ATT CAT GGT CTG GCT CAC CCT GAA ATG GGT CAT GGT ATT CCG ATC CGT CAT CCT GAT
      Pro Ile His Gly Leu Ala His Pro Glu Met Gly His Gly Ile Pro Ile Arg His Pro Asp

 638  GAT CGT GAT TTC GAA GGT TGC TGC CCT TAT CAT GGC GGT TGC TAT GAA GGT CTC GCC AGC
      Asp Arg Asp Phe Glu Gly Cys Cys Pro Tyr His Gly Gly Cys Tyr Glu Gly Leu Ala Ser

 698  GGC ACG GCT ATC CGT AAA CGT TGG GGC AAG GCT CTG AAC GAA ATG GAA CCG GCC GAA TTT
      Gly Thr Ala Ile Arg Lys Arg Trp Gly Lys Ala Leu Asn Glu Met Glu Pro Ala Glu Phe

 758  GAA AAA GCC AGA GAA ATC ATT GCT TTC TAT TTG GCT CAC TTC AAC GTC ACG CTT CAG GCC
      Glu Lys Ala Arg Glu Ile Ala Ala Phe Tyr Leu Ala His Phe Asn Val Thr Leu Gln Ala

 818  TTT ATT TCA CCG GAA CGT ATT GTT TTC CGC CGC GGC GTT ATG CAT GTT GAC GGT ATG CTG
      Phe Ile Ser Pro Glu Arg Ile Val Phe Gly Gly Gly Val Met His Val Asp Gly Met Leu

 878  GCT TCT GTT CGT CGT CAG ACG GCT GAA ATT GCC AAT AGT TAT TTT GAA GGT GCT GAC TTC
      Ala Ser Val Arg Arg Gln Thr Ala Glu Ile Ala Asn Ser Tyr Phe Glu Gly Ala Asp Phe

 938  GAA AAA ATT ATC GTA TTA CCA GGG TTA GGT GAT CAG GCC GGT ATG ATG GGT GCC TTC GCC
      Glu Lys Ile Ile Val Leu Pro Gly Leu Gly Asp Gln Ala Gly Met Met Gly Ala Phe Ala

 998  TTG GCA TTG GCT GCA GAA AAT AAA TAA TGTGGATAAATCTTCCAAAAATCAATTTTCTTAACCGAAAAGG
      Leu Ala Leu Ala Ala Glu Asn Lys ***

1068  GATTTTGGAAAAT
```

FIG. 2. Sequence analysis of the first 1,080 bases of the insert in plasmid pZFK2, including the open reading frame encoding fructokinase commencing at base 119. The N-terminal amino acid sequence confirmed most of the amino acids 1 to 20 shown in italics. The putative transcription terminator is shown at the 3′ end underlined.

```
TGKESGNYLAIDLGGTNLRVVLVKLGGDRTFDTTQSYKLPHDMRTTKHQEELN...  YE
MKNDKKIYGCIEGGGTKFMLALIDSDRKMLAVERVPTTTPEETLGKSVEFFKKALP  FK
    MEIVAIDIGGTHARFSIAEVSNGRVLSLGEETTFKTAEHASLQLAWERFGE   GK

QYADSFA SFGIASFGPLCLDRKSPKWGYITNTPKPFWPNTDVVTPFKEAFGCPVE  FK
KLGRPLPRAAAIAWAGPVHGEVLKLTNNPWVLRPATLNEKLDIDTHVLINDFGAVA  GK

IDTDVNGAALAENFWGASKGT HTSVYVTVGTGFGGGVL                   FK
HAVAHMDSSYLDHICGPDEALPSDGVITILGPGTGLGVAHLLRTEGRYFVIETEGG  GK

 IDGKPIHGLAHPEMGHGI        PIRHPDDRDFEGCCPYHGGCYEGLASGTAI  FK
HIDFAPLDRLEDKILARLRERFRRVSIERIISGPGLGNIYEALAAIEGVPFSLLDD  GK

RKRWGKALNEMEPAEFEKAREIIAFYLAHFNVTLQAFISPERIVFGGGVMHVDGML  FK
IKLWQMAL EGKDNLAEAALDRFCLSLGAIAGDLALAQGRTSVVIGGGVGLRIASH  GK

ASVRRQTAEIANSYFEGADFEKIIVLPGLGDQAGMMGAFALALAAENK          FK
LPESGFRQRFVSKGRFERVMSKIPVKLITTPQPGLLGAQLPMPTNILKLNNIF     GK
```

FIG. 3. Comparison of the glucokinase and fructokinase sequences, allowing only four gaps in the fructokinase sequence (two of which are single amino acids) and a single amino acid gap in the glucokinase sequence. The sequence of *S. cerevisiae* (YE) hexokinase A (17), commencing at amino acid 74, which is part of the ATP-binding site (2), is given on the top line and compared with the glucokinase (GK) sequence. |, identity; :, similarity. There are 49 identical amino acids in this alignment of glucokinase and fructokinase (FK), which is 16% of the fructokinase residues.

a 1.2-kb transcript (Fig. 4). If the transcript does indeed terminate at the putative terminator (bp 1080 of Fig. 2), then the *frk* promoter and transcriptional initiation site lie approximately 250 bp upstream of the start codon. Therefore, it is unlikely that the *frk* promoter is present on pZFK1 (Fig. 1).

Quantitation of the *frk* mRNA levels in *Z. mobilis* cells grown on fructose or on glucose was performed in order to determine whether the elevation in enzyme activity was the result of transcriptional regulation (Fig. 4). The level of *frk* mRNA was threefold higher in fructose-grown cells than on glucose-grown cells. In order to determine whether the increased level of transcript in fructose-grown cultures was the result of differential transcript stability, the half-life of the *frk* mRNA was determined in fructose and glucose cultures. Decay of the transcript in both cultures followed a similar pattern, beginning just 3 min after rifampin addition. The measured half-life of the *frk* transcript in fructose-grown cells was 6.6 ± 0.6 min, while that in glucose-grown cells was 6.2 ± 0.5 min. Thus, mRNA stability does not appear to play a role in regulating the levels of the *frk* message.

DISCUSSION

Hexose-phosphorylating enzymes from a number of sources have now been sequenced. These include the eukaryotic hexokinases from rat brain (25), the two major isoenzymes from *S. cerevisiae* (17, 28), rat liver glucokinase

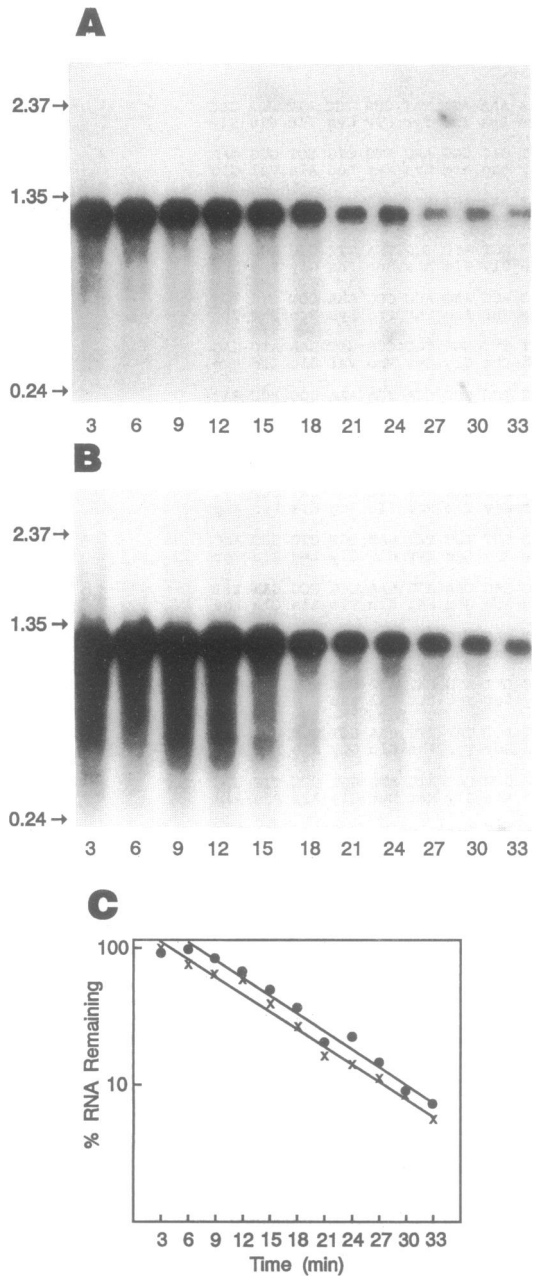

FIG. 4. (A and B) Northern blot analysis of the *Z. mobilis frk* transcript with a gene-specific hybridization probe. Total RNA was harvested from log-phase cultures, growing on glucose (A) or fructose (B), after inhibition of transcription by rifampin at the indicated times (in minutes). The gels were loaded with 2.5 μg of RNA per lane. Size markers (in kilobases) are provided on the left. (C) Semilogarithmic plot of the data shown in panels A (●) and B (x), quantitated by scanning of the filters with the Ambis system. The decay rate of the *frk* mRNA in the glucose-grown culture was 6.2 ± 0.5 min, whereas in the fructose-grown culture, it was 6.6 ± 0.6 min.

(2), *Z. mobilis* sugar-specific glucokinase (4), and fructokinase (this work). In addition, many other such enzymes have been isolated and characterized (8, 13, 23, 24). In general, eukaryotic enzymes have subunit molecular masses of 50 to 55 kDa (yeast hexokinase, liver glucokinase), or 100 kDa (mammalian hexokinases), whereas prokaryotic enzymes are mostly smaller, at 30 to 35 kDa; in most cases, the mature enzyme is dimeric.

Comparison of the eukaryotic enzymes indicates extensive homology among them, especially in the areas identified (from the *S. cerevisiae* hexokinase crystal structure) as ATP- and glucose-binding sequences (5, 17). It was estimated that approximately two-thirds of the amino acids were identical or similar in these comparisons (2, 19). In contrast, *Z. mobilis* glucokinase showed only 21% identity with *S. cerevisiae* hexokinase (allowing for the size difference) and 21% similarity, enough to suggest a common origin (4). *Z. mobilis* fructokinase shows even less homology and surprisingly little similarity with *Z. mobilis* glucokinase. There does seem to be a convincing region of homology with the *S. cerevisiae* hexokinase ATP-binding site (2), close to the N terminus of both fructokinase and glucokinase.

Few fructose-specific phosphorylating enzymes have been characterized; the liver enzyme is well-known, but it phosphorylates at position 1 compared with position 6 for fructose 6-kinase described here. Several enzymes that phosphorylate fructose and mannose have been reported, but not completely purified (23, 24). Although glucose and fructose are not highly similar in three-dimensional shape, *S. cerevisiae* hexokinase (and, at sufficiently high fructose concentrations, liver glucokinase [6]) phosphorylates both of them. Thus, one would expect that the sugar-specific enzymes found in prokaryotes could have evolved from a common hexokinase. The comparison between the two *Z. mobilis* enzymes shown in Fig. 3 shows few similarities; convergent evolution of function rather than structure may be considered more likely. As this manuscript was finalized, the sequences of the *scrK* genes (encoding fructokinase) of *E. coli* and *Klebsiella pneumoniae* were published (3). Although the lengths of the encoded proteins are similar (305 compared with 302 residues), data base comparison did not show any significant homology.

Sequence analysis of the *frk* gene indicated an open reading frame of 906 bp (including the initiator methionine) that encodes a 32,572-Da protein. This is somewhat larger than the 28-kDa subunit estimated from SDS-polyacrylamide gel electrophoresis (28), which is probably a reflection on the nature of the standard proteins used for calculating the molecular size, e.g. ovalbumin and carbonic anhydrase; several *Z. mobilis* enzymes have given 2-5 kDa lower values on SDS polyacrylamide gels than the values deduced from subsequent gene sequencing (4, 18). The initiator methionine is retained on the mature fructokinase protein of 13 *Z. mobilis* enzymes for which we have determined the N-terminal amino acid sequence, retention of terminal methionine occurs only in fructokinase, glucokinase, and alcohol dehydrogenase-1.

The putative ribosome-binding sequence, GAGUGG, is of poorer quality than that of other *Z. mobilis* genes that encode the enzymes involved in central metabolism (7). However, the region between the start codon and ribosome-binding site on the mRNA is AU rich, indicating a potential for efficient translation (12). The *frk* gene is followed by a large stem-loop structure that might serve as a transcriptional terminator (31).

Northern blot analysis showed that the *frk* gene is encoded on a 1.2-kb transcript, indicating that *frk* is monocistronic. Given the length of the transcript and the location of the putative terminator, it is likely that transcription of *frk* begins 250 bp upstream of the start codon in a region that is not present on pZFK1 and not sequenced in this study. 5′ untranslated regions (5′UTR) of 200 bases or more are not

uncommon in *Z. mobilis* (15). The significance of these lengthy 5'UTR in *Z. mobilis* is not known; in the case of *frk*, it does not appear to play a role in stabilization of the transcript. The *frk* 5'UTR is approximately 175 bases longer than that of *pgi*, but the two transcripts have virtually identical half-lives (average, 6.3 min [15]).

The results of this study indicate that the elevation in fructokinase activity that is observed when *Z. mobilis* is grown on fructose instead of glucose is the result of an increased rate of transcription. The alternative possibility that this was the result of differential mRNA stability was ruled out by measuring the decay rates of the *frk* transcript in the two cultures and finding them to be the same. The activity increase was only 1.9-fold, compared with 3-fold for the mRNA level increase; a virtually identical result was obtained for the *pgi* gene (15), but not for other enzymes involved in central metabolism. It must be concluded that growth on fructose causes an increase in transcription of only those genes (i.e., *frk* and *pgi*) that are uniquely required for fructose metabolism. Although both enzymes are present when *Z. mobilis* cells are grown on glucose, their amounts are not quite enough to allow sufficient metabolic flux from fructose to glucose 6-phosphate, if they are suddenly switched to fructose as the sugar source. The lower rate of production of glucose 6-phosphate and therefore total glycolysis in these circumstances would not produce enough ATP to allow the cells to grow without constraint (1). The regulation of these two constitutively expressed enzymes is interesting. The molecular aspects of regulation shared by *frk* and *pgi* are currently being investigated.

REFERENCES

1. **Algar, E. M., and R. K. Scopes.** 1985. Studies on cell-free metabolism: ethanol production by extracts of *Zymomonas mobilis*. J. Biotechnol. **2**:275–287.
2. **Andreone, T. L., R. L. Printz, S. J. Pilkis, M. A. Magnuson, and D. K. Granner.** 1989. The amino acid sequence of rat liver glucokinase deduced from cloned cDNA. J. Biol. Chem. **264**: 363–369.
3. **Aulkemeyer, P., R. Ebner, G. Heilmann, K. Jahreis, K. Schmid, S. Wrieden, and J. W. Lengler.** 1991. Molecular analysis of two fructokinases involved in sucrose metabolism of enteric bacteria. Mol. Microbiol. **5**:2912–2922.
4. **Barnell, W. O., K. C. Yi, and T. Conway.** 1990. Sequence and genetic organization of a *Zymomonas mobilis* gene cluster that encodes several enzymes of glucose metabolism. J. Bacteriol. **172**:7227–7240.
5. **Bennett, W. S., and T. A. Steitz.** 1980. Structure of a complex between yeast hexokinase A and glucose. J. Mol. Biol. **140**:211–230.
6. **Cardenas, M. L., E. Rabajille, and H. Niemeyer.** 1984. Fructose is a good substrate for rat liver 'glucokinase' (hexokinase D). Biochem. J. **222**:363–370.
7. **Chen, E. Y., and P. H. Seeburg.** 1985. Supercoil sequencing: a fast and simple method of sequencing plasmid DNA. DNA **4**:165–170.
8. **Delvalle, J. A., and C. Asencio.** 1978. Distribution of ATP-dependent hexose kinases in microorganisms. BioSystems **10**: 265–282.
9. **Dimarco, A. A., and A. H. Romano.** 1985. D-Glucose transport system of *Zymomonas mobilis*. Appl. Environ. Microbiol. **49**: 151–157.
10. **Doelle, H. W.** 1982. Kinetic characteristics and regulatory mechanisms of glucokinase and fructokinase from *Zymomonas mobilis*. Eur. J. Appl. Microbiol. Biotechnol. **14**:241–246.
11. **Doelle, H. W.** 1982. The existence of two separate constitutive enzymes for glucose and fructose in *Zymomonas mobilis*. Eur. J. Appl. Microbiol. Biotechnol. **15**:20–24.
12. **Gold, L., and G. Stormo.** 1987. Translation initiation, p. 1302–1307. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
13. **Goward, C. R., R. Hartwell, T. Atkinson, and M. D. Scawen.** 1986. The purification and characterization of glucokinase from the thermophile *Bacillus stearothermophilus*. Biochem. J. **237**: 415–420.
14. **Gunasekaran, P., T. Karunakaran, B. Cami, A. G. Mukundan, L. Preziosi, and J. Baratti.** 1990. Cloning and sequencing of the *sacA* gene: characterization of a sucrase from *Zymomonas mobilis*. J. Bacteriol. **172**:6727–6735.
15. **Hesman, T., W. O. Barnell, and T. Conway.** 1991. Cloning, characterization, and nucleotide sequence analysis of a *Zymomonas mobilis* phosphoglucose isomerase gene that is subject to carbon source-dependent regulation. J. Bacteriol. **173**: 3215–3223.
16. **Innis, M. A., K. B. Myambo, D. H. Gelfand, and M. A. D. Brow.** 1988. DNA sequencing with *Thermus aquaticus* DNA polymerase and direct sequencing of polymerase chain reaction-amplified DNA. Proc. Natl. Acad. Sci. USA **85**:9436–9440.
17. **Kopetzki, E., K.-D. Entian, and D. Mecke.** 1985. Complete nucleotide sequence of the hexokinase P1 gene (HXK1) of *Saccharomyces cerevisiae*. Gene **39**:95–102.
18. **Mackenzie, K. F., T. Conway, H. C. Aldrich, and L. O. Ingram.** 1989. Expression of *Zymomonas mobilis adhB* (encoding alcohol dehydrogenase II) and *adhB-lacZ* operon fusions in recombinant *Zymomonas mobilis*. J. Bacteriol. **171**:4577–4582.
19. **Marcus, F., and T. Ureta.** 1986. Amino acid sequence homology between yeast hexokinases and rat hexokinase C. Biochem. Biophys. Res. Commun. **139**:714–719.
20. **Mortatte, M. P. L., H. H. Sato, and Y. K. Park.** 1983. Induction and some characteristics of sucrose hydrolyzing enzyme from *Zymomonas mobilis*. Biotechnol. Lett. **5**:229–232.
21. **Neale, A. D., R. K. Scopes, R. E. H. Wettenhall, and N. J. Hoogenraad.** 1987. Pyruvate decarboxylase of *Zymomonas mobilis*: isolation, properties, and genetic expression in *Escherichia coli*. J. Bacteriol. **169**:1024–1028.
22. **O'Mullan, P., M. Szakacs-Dobozi, and D. E. Eveleigh.** 1991. Identification of saccharolytic enzymes of *Zymomonas mobilis* CP4. Biotechnol. Lett. **13**:137–142.
23. **Porter, E. V., B. M. Chassy, and C. E. Holmlund.** 1980. Partial purification and properties of a mannofructokinase from *Streptococcus mutans* SL-1. Infect. Immun. **30**:43–50.
24. **Sapico, V., and R. L. Anderson.** 1967. An adenosine 5'-triphosphate: hexose 6-phosphotransferase specific for D-mannose and D-fructose from *Leuconostoc mesenteroides*. J. Biol. Chem. **242**:5086–5092.
25. **Schwab, D. A., and J. E. Wilson.** 1989. Complete amino acid sequence of rat hexokinase, deduced from the cloned cDNA, and proposed structure of a mammalian hexokinase. Proc. Natl. Acad. Sci. USA **86**:2563–2567.
26. **Scopes, R. K.** 1977. Purification of glycolytic enzymes by affinity elution chromatography. Biochem. J. **161**:253–263.
27. **Scopes, R. K., V. Testolin, A. Stoter, K. Griffiths-Smith, and E. M. Algar.** 1985. Simultaneous purification and characterization of glucokinase, fructokinase and glucose 6-phosphate dehydrogenase from *Zymomonas mobilis*. Biochem. J. **228**:627–634.
28. **Stachelek, C., J. Stachelek, J. Swan, D. Bostein, and W. Konigsberg.** 1986. Identification, cloning and sequencing of hexokinases A and B from yeast. Nucleic Acids Res. **14**:945–963.
29. **Swings, J., and J. De Ley.** 1977. The biology of *Zymomonas*. Bacteriol. Rev. **41**:1–46.
30. **Viikari, L., and M. Korhola.** 1986. Fructose metabolism in *Zymomonas mobilis*. Appl. Microbiol. Biotechnol. **24**:471–476.
31. **Yager, T. D., and P. H. von Hippel.** 1987. Transcript elongation and termination in *Escherichia coli*, p. 1241–1275. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M.

Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.

32. **Yanase, H., H. Fukushi, N. Ueda, Y. Maeda, A. Toyoda, and K. Tonomura.** 1991. Cloning, sequencing and characterization of the intracellular invertase gene from *Zymomonas mobilis*. Agric. Biol. Chem. **55:**1383–1390.
33. **Zachariou, M., and R. K. Scopes.** 1986. Glucose-fructose oxidoreductase, a new enzyme isolated from *Zymomonas mobilis* that is responsible for sorbitol production. J. Bacteriol. **167:**863–869.