Cloning of the Gene and Amino Acid Sequence for Glucose 6-Phosphate Dehydrogenase from *Leuconostoc mesenteroides*

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Amino acid sequencing of glucose 6-phosphate dehydrogenase (Glc6PD) from *Leuconostoc mesenteroides* yielded sequence for over 75% of the protein. Two oligonucleotides based on the amino acid sequence were used to isolate a partial Glc6PD gene clone (pLMzN65), from a pUC9 library, containing 85% of the coding sequence and the 5'-untranslated DNA, but lacking the 5'-noncoding DNA sequence and the portion of the gene encoding the 65 N-terminal amino acids. Attempts to obtain a full-length clone from λ libraries were unsuccessful, possibly due to restriction of *L. mesenteroides* DNA by *Escherichia coli* host cells. The 5'-untranslated DNA was amplified by the polymerase chain reaction and partially sequenced. To obtain unmodified DNA for the gene, oligonucleotides corresponding to the 5'- and 3'-noncoding sequences were used to amplify the gene by the polymerase chain reaction, and a 1.8-kilobase pair fragment was isolated and cloned into pUC19. The recombinant plasmid, pLMz, contains the entire Glc6PD gene and expresses the gene in *E. coli*. pLMz was sequenced showing that the enzyme consists of 485 amino acids. *L. mesenteroides* Glc6PD is 31% identical to the human enzyme.

Glucose 6-phosphate dehydrogenase (Glc6PD) from *Leuconostoc mesenteroides* is one of a few dehydrogenases that can utilize either NAD⁺ or NADP⁺ as coenzyme (1). In view of the specificity of the great majority of dehydrogenases for either NAD⁺ or NADP⁺, and the distinct metabolic roles of these two coenzymes, those structural and regulatory features that enable a dehydrogenase to utilize both nicotinamide coenzymes are of unique interest. Our previous studies with *L. mesenteroides* Glc6PD (summarized in Ref. 2) have identified critical amino acid residues of this enzyme; shown that the kinetic mechanisms of the NAD- and NADP-linked reaction differ, allowing for differential control of the two reactions; demonstrated that NAD⁺ and NADP⁺ induce substantially different conformational changes of the enzyme; and shown that these coenzymes themselves assume nonidentical conformations when they bind to the enzyme.

The *Escherichia coli* (3), *Zymomonas mobilis* (4), *Drosophila melanogaster* (5), rat liver (6), and human (7) Glc6PD genes have been cloned, and the amino acid sequences of the corresponding enzymes have been deduced. This paper describes the cloning of the *L. mesenteroides* Glc6PD gene and the amino acid sequence of the enzyme, derived from partial sequencing of the protein and complete sequencing of the DNA. This sequence is compared with those of *E. coli* and human Glc6PDs, which utilize NADP⁺ specifically (8, 9), and that of *Z. mobilis* Glc6PD, which can utilize either NADP⁺ or NAD⁺ (10). Those structural features of the *L. mesenteroides* Glc6PD that allow for NAD⁺ binding and utilization can now be probed using site-directed mutagenesis studies. This information will be augmented by studies on the three-dimensional structure of the enzyme, which are currently in progress (11).

The cloning of the *L. mesenteroides* Glc6PD gene was described previously by another group (12). No sequence information was presented in that paper, and the results of the cloning presented here differ from those reported by Murphy et al. (12).

EXPERIMENTAL PROCEDURES

Materials—Restriction enzymes, DNA modifying enzymes, pUC9, pUC19, M13mp18, and M13mp19 were from BRL. Taq polymerase was from Perkin-Elmer Cetus, modified T₇ DNA polymerase (Sequenase) from U. S. Biochemical Corp., TPCK-treated trypsin from Sigma, radiolabeled nucleotides from Du Pont-New England Nuclear, Genedine from BTO 101, AGM11 from Promega, and the ECL gene detection system from Amersham Corp. Calf intestine alkaline phosphatase, endoprotease-Arg C, endoproteinase-Lys C, and endoproteinase-Glu C were from Boehringer Mannheim. *L. mesenteroides* Glc6PD and thermolysin were obtained from Worthington. All other reagents were analytical grade.

Bacterial Strains—*L. mesenteroides* was from the American Type Culture Collection (ATCC 12291). *E. coli* strains DH5α and DH5αF⁺ were provided by Dr. David T. Sullivan (Syracuse University). DH5αMCR was from BRL, and MB406 and LE392 were from Promega.

Amino Acid Sequencing—All amino acid sequencing was performed using Glc6PD obtained commercially. The first step in amino acid sequencing involved CNBr cleavage. The protein was subjected to CNBr (1 mg/ml in 70% formic acid) for 24 h and then lyophilized three times. The resulting fragments were separated by gel filtration on 30-cm Bio-Rad TSK 250/TSK 125 columns, in tandem, using 3 M guanidine HC1, 50 mM sodium phosphate, 10 mM 2-mercaptoethanol, pH 7.0, at 1.0 ml/min isocratically. Five major peaks were collected, dialyzed against double-distilled H₂O twice in 1000 molecular weight cut-off tubing, and lyophilized. Portions of these fractions were run on a Waters μBondapak C18 column using various gradients (mostly 25–65% or, occasionally, 25–75% B, over 80–100 min, where
A was 0.1% trifluoroacetic acid and B was 0.1% trifluoroacetic acid in 80% acetonitrile.

Maleylated peptides were generated as follows. The protein was dissolved in 2.0 ml of 0.1 M sodium pyrophosphate buffer, pH 9.0, and treated with 1.0 M maleic anhydride in redistilled dioxane, added in 10% increments, and treated with 1.0 M maleic anhydride in redistilled dioxane, added in 10% increments. During the reaction the pH was maintained at 9.0 with 0.1 M NaOH. Tryptic digestion of the enzyme was formed in 0.1 M ammonium bicarbonate at 37 °C for 4 h. The product was lyophilized and run on a Waters µBondapak column under the same conditions as described above.

To prepare thermolysin peptides, Glc6PD was dissolved in 100 mM NH4HCO3 and 5 mM CaCl2 in the presence of thermolysin at a concentration of 2% enzyme/substrate (w/w). Incubation proceeded at 37 °C for at least 4 h. The digest was run on a Waters µBondapak reverse phase C18 column, at a flow rate of 1.5 ml/min, with 0-75% B over 150 min, where buffer A was 0.1% trifluoroacetic acid and buffer B was 0.1% trifluoroacetic acid in acetonitrile. Some peptides were further purified using a Beckman Ultrasphere-ODS reverse phase C18 column and an ammonium acetate gradient system, where buffer A was 10 mM ammonium acetate, pH 6.9, and buffer B was 20 mM ammonium acetate/acetonitrile 50:50; the gradient was 0-75% B over 75 min at a flow rate of 1.0 ml/min.

Additional peptides were generated using endoproteinase-Lys C. The protein was dissolved in 0.1 M ammonium bicarbonate buffer, pH 9.0, and endoproteinase-Lys C was added at a ratio of 1:100. Incubation proceeded at 37 °C for 1-2 h, after which another aliquot of endoproteinase-Lys C was added, and incubation was continued overnight. The mixture was then applied to a Hilar reverse phase C18 column, and fractions were eluted using a trifluoroacetic acid gradient of 25-100% B over 70 min.

Digestions with endoproteinase-Arg C were similar to those for endoproteinase-Lys C, except that the pH was 8.0-8.5 and the ratio used was 1:50.

Endoproteinase-Glu C digestions were performed in 50 mM sodium phosphate, pH 7.8, using a ratio of 1:100. The mixture was separated on a C18 column using a trifluoroacetic acid gradient, 20-100% B over 90 min, at a flow rate of 1.0 ml/min, where buffer A was 0.1% trifluoroacetic acid and buffer B was 0.1% trifluoroacetic acid in 80% acetonitrile.

Purified peptides were sequenced by automated Edman degradation on an Applied Biosystems 470A gas-phase micro-sequinator.

Oligonucleotides—Oligonucleotides 1 (5'-GCTTTYTCRAARTC-RTTYTC 3') and 2 (5'-CCARAANACCCACGRTC 3'), where N is A, G, T, or C, R is A or G, and Y is C or T, were synthesized at Queen University's, on the complementary sequences to END-FENA (amino acids 161-167) and DAVFVF (amino acids 479-484) respectively. Oligonucleotides 3 (5'-TACCGTGTACAGCAAGATGAAATGCTTTATTTCTGAAC 3'), 4 (5'-CTGGTGTGCCTGTATGGGTGTC 3'), and 5 (5'-GGGGATCCCATCTA-AAAGCTACTTCA 3') were synthesized at the Syracusa University DNA and Protein Core Facility. Oligonucleotide 3 is complementary to the C-terminal amino acid sequence VSEIKTLVTFFGGTGD using the best codon frequencies from the DNA sequence of the enzyme.

Polymerase Chain Reaction—The polymerase chain reaction was done on an Eppendorf Twinblock System for 30 cycles, each cycle consisting of 1 min at 94 °C, 1 min at 42 °C, and 5 min at 68 °C (17).

RESULTS

Amino Acid Sequencing—Figs. 1-4 and Table I exemplify the approach taken toward deriving the amino acid sequence of L. mesenteroides Glc6PD. Sequencing of the native enzyme yielded the first 50 residues (Table I, Fig. 1). CNBr cleavage of native Glc6PD followed by gel filtration and reverse phase HPLC yielded the sequences for CNBr peptides 3, 5, 7, 9, 12, 13, and 15 as shown in Table I and Fig. 6. An example of the purification procedure used for each of these peptides is shown in Figs. 1 and 2. Other sequences were obtained by enzymatic cleavage of the native protein using a variety of proteolytic enzymes as shown in Table I. A typical example of the results obtained is seen in Figs. 3 and 4. Using this approach, large portions of contiguous and overlapping sequence were obtained, particularly at the N and C termini. A total of 371 residues, approximately 76% of the total, were sequenced in this manner. However, several gaps remained, and because of the difficulty of isolating and sequencing peptides from the central portion of the enzyme (residues 320-374), it was decided to use the amino acid sequence data for the construction of oligonucleotide probes and to derive the amino acid sequence from the DNA sequence of the cloned Glc6PD gene.

Cloning of the Glucose 6-phosphate Dehydrogenase Gene—The amplified L. mesenteroides pUC9 library was screened using as probes oligonucleotides 1 and 2. After an initial Southern Blots—Southern blots were done using the ECL gene detection system except when screening with oligonucleotide probes by the method of Wallace and Miyada (13).

Construction and Screening of L. mesenteroides Libraries—L. mesenteroides genomic DNA was isolated by the method of Murphy et al. (12). Restriction fragments of 3.5-10.0 kb were isolated (using Gene cleaner) from a MboI partial digest and ligated to BamHI-cut, phosphatase-treated pUC9. The ligation mixture was used to transform E. coli strain DH5α. Recombinant plasmids from transformed cells were separated from ligated pUC9 by gel electrophoresis, isolated (using Gene cleaner), and transformed back into DH5α. The library was screened with oligonucleotides 1 and 2 by the method of Wallace and Miyada (13).

Genomic libraries in XGEM11 were made by ligating electroeluted 14-20-kb fragments of an MboI partial digest to a BamHI λ arms. The λ libraries were screened by the method of Wahl and Berger (14). DNA Techniques—Isolation of plasmids (15) and plasmid transformations (16) were performed as described. The procedures of Sambrook et al. (17) were used for standard DNA protocols.

DNA Sequencing—DNA sequencing was done by the method of Sanger et al. (18) on single-stranded M13 subclones or on double-stranded DNA using either the Klonef or Sequenase.

Assays of Glc6PD Activity—Enzyme activity was measured at 25 °C by following the appearance of NADPH at 340 nm. Assays were initiated by the addition of enzyme to 1.0 ml of solution containing 33 mM Tris-Cl (pH 7.6), 1.6 mM glucose 6-phosphate, and 114 μM NADP.

Crude Extracts of Glc6PD from E. coli—Cells from overnight cultures of E. coli were pelleted in 1.5-ml microfuge tubes, resuspended in 400 μl of buffer (8% sucrose, 10 mM Tris-Cl, pH 8.0, 50 mM EDTA, pH 8.0, and 0.5% Triton X-100), and incubated at 37 °C for 30 min after the addition of 50 μl of lysozyme (10 mg/ml in 10 mM Tris-Cl, pH 8.0, 1.0 mM EDTA, pH 8.0). Cellular debris was pelleted for 5 min in a microfuge, and the supernatant was assayed for enzyme activity.

Fig. 1. Gel filtration on HPLC of CNBr-treated native enzyme. See “Experimental Procedures.” A UFS, absorbance units at full scale.
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Fig. 2. Further processing of peak 5 from Fig. 1 by reverse phase HPLC. See “Experimental Procedures.” Peak B was sequenced and gave the sequence for amino acids 423-445. AUFS, absorbance units at full scale.

Fig. 3. HPLC profile of endoproteinase-Glu C-treated native Glc6PD. See “Experimental Procedures.” AUFS, absorbance units at full scale.

Fig. 4. Purification by reverse phase HPLC of peak 22 from E. coli strain MB406. See “Experimental Procedures.” Peak 22/10 (peptide 8 in Table I and Fig. 6) represents amino acids 236-246, and peak 22/13 (peptide 11 in Table I and Fig. 6) represents amino acids 304-319. AUFS, absorbance units at full scale.

To obtain a full-length clone two λGEM11 libraries were screened. One library in E. coli strain MB406 was screened with the EcoRI-SphI fragment of pLmzΔN65. Characterization of the clones isolated from this library revealed that none contained the DNA encoding the N-terminal amino acids. The other library, in E. coli strain LE392, was screened with the EcoRI-SphI fragment and oligonucleotide 3. No clones were isolated that hybridized to oligonucleotide 3, although this probe did hybridize to L. mesenteroides genomic DNA on a Southern blot (data not shown). A possible explanation for the difficulty in cloning the 5' end of the Glc6PD gene is that the L. mesenteroides DNA may contain modified DNA sequences that are cleaved by E. coli restriction enzyme systems present in the host strains used.

Southern analysis of L. mesenteroides DNA by the EcoRI-SphI fragment and oligonucleotide 3 gave a partial map of restriction sites 5' to the start of the gene (data not shown). An NsiI site located approximately 2.7 kb 5' to the expected start of the gene was found. NsiI-SphI digests of L. mesenteroides genomic DNA produced 4.4-kb fragments containing the Glc6PD gene that were isolated by electrophoresis, ligated to pUC19, and transformed into E. coli strain DH5αMCR, which lacks three known restriction enzymes that cleave methylated DNA. The transformed colonies were screened using the EcoRI-SphI fragment. Unfortunately, no clones containing the Glc6PD gene were isolated from this screen either.

To obtain unmodified DNA of the gene, 4.5-kb NsiI-ScaI fragments containing the entire Glc6PD gene were isolated by electrophoresis and ligated to pUC19. The recombinant plasmids were linearized and then amplified by PCR using as primers oligonucleotide 4, which is complementary to the 3'-untranslated DNA sequence, and the reverse sequencing primer, which is complementary to sequences present in pUC19. No PCR product was observed, presumably due to the length (4.4 kb) of the desired product. Therefore, oligonucleotide 3, previously used to screen a λ library, and the reverse primer were used to amplify by PCR the 5'-untranslated region to the Glc6PD gene. A 2.7-kb PCR product was isolated by electrophoresis and partially sequenced using oligonucleotide 3 as the primer. Oligonucleotide 5, based on the DNA sequence of this 2.7-kb PCR product and containing an engineered BamHI site, and oligonucleotide 4 were used in a
PCR on L. mesenteroides genomic DNA to amplify the entire gene. A 1.8-kb PCR fragment was isolated, digested by BamHI and SphI, ligated to pUC19, and transformed into DH5αMCR. Transformed colonies containing the recombinant plasmid (pLmz) had Glc6PD activity that could utilize either NADP⁺ or NAD⁺. The NAD⁺ reaction had 1.8 times the specific activity of the NADP⁺ reaction under conditions which corresponds well to the experimental value of 54,800 (24). The deduced isoelectric point of 4.7 also corresponds to the experimental value of 4.6 (25). Fig. 7 shows the sequence identity among the human, E. coli, Z. mobilis, and L. mesenteroides Glc6PDs. The human (9) and E. coli (8) enzymes only utilize NADP⁺ under physiological conditions, whereas the Glc6PDs from Z. mobilis (10) and L. mesenteroides (1) can utilize either NADP⁺ or NAD⁺. There is approximately 30–36% sequence identity between any two of these Glc6PDs. A region of total identity is seen, beginning at Arg-175 in L. mesenteroides Glc6PD sequence. The identical sequence occurs in Glc6PDs from rat liver (6), D. melanogaster (5), Pichia jadinii (26), and Saccharomyces cerevisiae (27). In the human (28), P. jadinii (26), and S. cerevisiae (27) Glc6PDs there is evidence that the Lys residue in this conserved region functions in glucose 6-phosphate binding.

There is 95% identity between the human and rat liver Glc6PDs (6) and 67% identity between human and D. melanogaster Glc6PDs (5). Human and rat liver Glc6PDs are inhibited by specific 17- and 20-keto steroids uncompetitively with respect to both NADP⁺ and glucose 6-phosphate (29). This steroid sensitivity of Glc6PD has been shown to be

**DISCUSSION**

The L. mesenteroides Glc6PD gene encodes a polypeptide of 485 amino acids. In confirmation of earlier studies (24), L. mesenteroides Glc6PD contains no Cys residues. The subunit molecular weight determined by the DNA sequence is 54,316, which corresponds well to the experimental value of 54,800 (24). The deduced isoelectric point of 4.7 also corresponds to the experimental value of 4.6 (25). Fig. 7 shows the sequence identity among the human, E. coli, Z. mobilis, and L. mesenteroides Glc6PDs. The human (9) and E. coli (8) enzymes only utilize NADP⁺ under physiological conditions, whereas the Glc6PDs from Z. mobilis (10) and L. mesenteroides (1) can utilize either NADP⁺ or NAD⁺. There is approximately 30–36% sequence identity between any two of these Glc6PDs. A region of total identity is seen, beginning at Arg-175 in L. mesenteroides Glc6PD sequence. The identical sequence occurs in Glc6PDs from rat liver (6), D. melanogaster (5), Pichia jadinii (26), and Saccharomyces cerevisiae (27). In the human (28), P. jadinii (26), and S. cerevisiae (27) Glc6PDs there is evidence that the Lys residue in this conserved region functions in glucose 6-phosphate binding.

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**TABLE I**

<table>
<thead>
<tr>
<th>Cleavage method (Peptide number)</th>
<th>Sequence</th>
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* N-terminal sequence of native protein.
* Specifically for R this cleavage was consistently at K. DNA sequence indicates R at position of "boldface" V; see "Discussion."
* Complete sequence of expected peptide.
* Incomplete sequence of expected peptide.
* Cleavage after D, not E. Specificity depends on buffer used during cleavage (19).
* DNA sequence indicates a D at "boldface" N/D; see "Discussion."
* Sequence beyond expected C-terminal R. Incomplete tryptic digestion.
* Cleavage did not occur at M but between D and P, presumably due to acidic conditions employed for CNBr cleavage (19).
* DNA sequence indicates a D at "boldface" N/D; see "Discussion."
* Did not cleave at N-terminal side of large hydrophilic residue but at A.
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**Fig. 5.** Restriction map of inserts of pLmzAN65 and pLmz.

A, the restriction map of the 5.3-kb insert of pLmzAN65, cloned into the *BamH I* site of pUC9. The *Sac I* site shown is one of several *Sac I* sites in the insert and is shown here because it was used for generating subclones for sequencing. The *Eco RI* site is in the pUC9 polylinker and is shown due to its use in experiments described in the text. Arrows indicate direction of DNA sequencing of subclones. The *BamH I* site, cloned into the *BamH I* and *Sph I* sites of pUC19. The *BamH I* site is located 5' to the start of the open reading frame, and the *Sph I* site is located 3' to the stop codon. The map shows some different restriction enzyme sites than the map in A to allow for a comparison to the restriction map in the paper of Murphy et al. (12) (see "Discussion" below). To orient the reader, the *NdeI*, *BgIII*, and *Sph I* sites in B and in the expanded map in A are identical. Arrows indicate direction of DNA sequencing of subclones. Abbreviations of restriction enzymes are as follows: *A*, *Ava I*; *Bg*, *BgIII*; *Br*, *BamH I*; *B*, *BstE II*; *Hc*, *HincII*; *Hd*, *HindIII*; *Hp*, *Hpa I*; *Nc*, *Nco I*; *Nd*, *Nde I*; *R*, *Eco RI*; *Rs*, *Rsa I*; *Sc*, *Sca I*; *Sp*, *Sph I*; and V, *Eco RV.*

directly responsible for the blocking action of specific steroids on cell differentiation (30). *D. melanogaster* Glc6PD is also NADP-specific, but preliminary studies show no specific steroid inhibition. 5. *L. mesenteroides* Glc6PD, like all non-mammalian Glc6PDs, is unaffected by steroids (20, 29). Steroid binding to rat mammary Glc6PD is believed to occur principally via hydrophobic amino acids (31), and presumably this is true for other mammalian Glc6PDs. The hydrophobicities of the human and *L. mesenteroides* Glc6PDs are similar (Fig. 8), the average hydrophobicities (32) being −0.36 for *L. mesenteroides* Glc6PD and −0.38 for the human enzyme.

The amino acid sequence based on protein sequencing was generally in good agreement with that derived from DNA sequencing, but two differences were consistently noted and are indicated in Table I. These differences are most likely attributed to the use of commercially obtained Glc6PD for amino acid sequencing, whereas DNA sequencing was performed on clones made from DNA isolated from an American Type Culture Collection strain of *L. mesenteroides*. The apparent presence, repeatedly observed, of two different amino acids in peptide 13 (Table I) may reflect microheterogeneity in the commercial preparation of Glc6PD.

The partial clone (pLmzAN65) was obtained using oligonucleotides based on amino acid sequences that were subsequently shown to be incorrect, due to difficulties in reading some sequences. Fortunately, these errors did not prevent the

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5 J. H. Williamson, personal communication.

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**Fig. 6.** DNA sequence and deduced amino acid sequence of Glc6PD. The DNA sequence was obtained from sequencing subclones from pLmzAN65 and pLmz. The *BamH I* and *Sph I* restriction sites of pLmz are underlined and italicized. The putative transcription promoter sequences (21) are shaded, and a possible transcriptional termination stem loop structure (22) is contained in the open box. The putative Shine-Delgarno domain in the ribosome binding site (23) is underlined. The start codon ATG is in boldface (the N-terminal amino acid of the protein is Val). The position of the last nucleotide in each line is indicated by the numbers with the A of the ATG initiation codon being nucleotide 1. The amino acid sequence is numbered in Fig. 7. The arrows under the first 50 amino acids correspond to amino acid sequencing results of the native protein. The numbered bars correspond to the peptides sequenced as shown in Table I.
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Table II
Codon usage from the L. mesenteroides Glc6PD gene

<table>
<thead>
<tr>
<th>Codon</th>
<th>Usage</th>
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<tbody>
<tr>
<td>Phe</td>
<td>UCC, UCG, UCA, UCU</td>
</tr>
<tr>
<td>Leu</td>
<td>UUA, UUG, CUU, CUA</td>
</tr>
<tr>
<td>Ile</td>
<td>AUU, AUC, ACA, ACC</td>
</tr>
<tr>
<td>Lys</td>
<td>AAA, AAG, CCA, CCG</td>
</tr>
<tr>
<td>Ser</td>
<td>UCA, UCG, AGU, AGC</td>
</tr>
<tr>
<td>Thr</td>
<td>ACU, ACC, AGC, AGG</td>
</tr>
<tr>
<td>Ala</td>
<td>GCC, GCG, GCA, GCT</td>
</tr>
</tbody>
</table>

DNA 5' to the start of the gene and the portion of the gene encoding the 65 N-terminal amino acids. By using PCR to amplify the gene with unmodified nucleotides, the E. coli could accept the L. mesenteroides Glc6PD gene. No mutations were detected in the PCR-amplified gene when comparing DNA sequences of pLmz with those of pLmzAN65. The expression of L. mesenteroides Glc6PD in E. coli may be due to the presence of the appropriate transcription and translation sequences in the 5'-untranslated DNA.

The previous paper (12) reporting the cloning of the L. mesenteroides Glc6PD did not include a sequence of the cloned gene. Murphy and co-workers presented a 3.4-kb restriction map and localized the gene to a 2.4-kb fragment. Their restriction map contains sites not present in pLmz, and the location and orientation of sites common to both restriction maps is not the same. The authors did not indicate the relative specific activities of the reactions with NAD+ and NADP+ (which were not measured by appearance of NADH or NADPH at 340nm) and presented no DNA or amino acid sequence information. The properties of the pLmz gene product described here are consistent with the physical properties of L. mesenteroides Glc6PD, its molecular weight, isoelectric point, amino acid sequence (including the absence of cysteine residues), and enzyme activity ratios with respect to NAD+ and NADP+, indicating that we have cloned the L. mesenteroides Glc6PD gene.

Determination of the sequence of L. mesenteroides Glc6PD and its expression in E. coli should enable us to utilize site-directed mutagenesis in order to elucidate structural features of the enzyme necessary for its dual nucleotide specificity. Comparison of these features with those that are characteristic for dehydrogenases that are specific for NAD+ or for...
NADP\textsuperscript{+} should help us to understand how these two classes of enzymes evolved.

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