**AFFINITY LABELING OF Lys-21 AND Lys-343**

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**Interaction of Leuconostoc mesenteroides Glucose-6-phosphate Dehydrogenase with Pyridoxal 5′-Diphospho-5′-adenosine**

Pyridoxal 5′-diphospho-5′-adenosine (PLP-AMP) inhibits glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides* competitively with respect to glucose 6-phosphate and noncompetitively with respect to NAD′ or NADP′, with $K_i = 40 \mu M$ in the NADP′-linked and 34 $\mu M$ in the NAD′-linked reaction. Incubation of glucose-6-phosphate dehydrogenase with [3H]PLP-AMP followed by borohydride reduction shows that incorporation of 0.85 mol of PLP-AMP per mol of enzyme subunit is required for complete inactivation. Both glucose 6-phosphate and NAD′ protect against this covalent modification. The proteolysis of the modified enzyme and isolation and sequencing of the labeled peptides revealed that Lys-21 and Lys-343 are the sites of PLP-AMP interaction and that glucose 6-phosphate and NAD′ protect both lysyl residues against modification. Pyridoxal 5′-phosphate (PLP) also modifies Lys-21 and probably Lys-343. Lys-21 is part of a highly conserved region that is present in all glucose-6-phosphate dehydrogenases that have been sequenced. Lys-343 corresponds to an arginyl residue in other glucose-6-phosphate dehydrogenases and is in a region that is less homologous with those enzymes. PLP-AMP and PLP are believed to interact with *L. mesenteroides* glucose-6-phosphate dehydrogenase at the glucose 6-phosphate binding site. Simultaneous binding of NAD′ induces conformational changes (Kurlandsky, S. B., Hilburger, A. C., and Levy, H. R. (1988) Arch. Biochem. Biophys. 264, 93-102) that are postulated to interfere with Schiff’s-base formation with PLP or PLP-AMP. One or both of the lysyl residues covalently modified by PLP or PLP-AMP may be located in regions of the enzyme undergoing the NAD′-induced conformational changes.

Glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides* can utilize either NAD′ or NADP′ as coenzyme (1, 2). The enzyme is a dimer consisting of identical subunits with a molecular weight of 54,800 (3). Earlier studies showed that PLP′ acts as an affinity probe for the Glc-6-P site (4, 5). PLP-AMP is an analog of PLP that was designed to have enhanced specificity for the nucleotide or coenzyme binding sites of kinases and dehydrogenases (6-8). We have labeled *L. mesenteroides* Glc-6-P-DH with PLP-AMP to ascertain whether this reagent could modify the coenzyme binding site. Here we report the sequences of two peptides which contain most of the incorporated label. We have also repeated the PLP labeling studies, showing that both PLP and PLP-AMP appear to interact with the same lysyl residues of the enzyme. Protection against covalent modification by PLP-AMP and PLP was afforded by both Glc-6-P and NAD′ but inhibition was competitive only with respect to Glc-6-P. We conclude that both PLP-AMP and PLP bind at the Glc-6-P site.

**EXPERIMENTAL PROCEDURES**

*Materials*—*L. mesenteroides* Glc-6-P-DH was obtained from Worthington Biochemicals. PLP-AMP and [3H]PLP-AMP were synthesized according to the method of Tamura et al. (7). [3H]PLP was synthesized by the method of Johansson et al. (9) as modified by Tamura et al. (7). NAD′, NADP′, and endoproteinase Lys-C came from Boehringer Mannheim; PLP and Glc-6-P were from Sigma. Aqueous counting scintillants were Liquiscint from National Diagnostics or BioSafe II from Research Products International Corp. Reversed-phase C4 analytical HPLC columns were purchased from J. T. Baker.

*Assays*—Glc-6-P-DH was routinely assayed at 25 °C by measuring the reduction of NADP′ at 340 nm. Assay solutions contained 57 $\mu M$ NADP′, 0.81 mM Glc-6-P, and 32 mM Tris-HCl, pH 7.8, and reactions were initiated by the addition of enzyme. Protein concentration was assayed using a modified Lowry procedure (10) with a standard curve constructed from the colorimetric response of known weights of defatted bovine serum albumin. Covalent incorporation of [3H]PLP-AMP was measured by the following procedure. An aliquot of column effluent containing a known quantity of enzyme was added to a 7-ml vial containing 1 ml of water, followed by addition of 100 $\mu l$ of 0.15% deoxycholate. After a 10-min incubation at room temperature, protein was precipitated by addition of 100 $\mu l$ of 72% trichloroacetic acid. Quantitative precipitation of the enzyme was verified experimentally, in agreement with observations with other proteins (10). Vials were centrifuged and the supernatant solutions were carefully aspirated. The pellets were dissolved in aqueous counting scintillant and counted in a Beckman LS 1800 scintillation counter.

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* The abbreviations used are: PLP, pyridoxal 5′-phosphate; Glc-6-P, glucose 6-phosphate; Glc-6-P-DH, glucose-6-phosphate dehydrogenase; HEPES, N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid; HPLC, high performance liquid chromatography; PLP-AMP, pyridoxal 5′-diphospho-5′-adenosine.
Kinetics—The kinetics of PLP-AMP inhibition were determined by incubating either 0.25 or 0.75 mM Glc-6-P-DH at 25 °C in the dark in 25 mM potassium phosphate, pH 7.6, either without PLP-AMP or with 10.5 or 31.6 µM PLP-AMP. Incubation mixtures included the varied substrate (Glc-6-P or coenzyme) at one of five concentrations, ranging from 0.5 to 5.0 times its K<sub>n</sub> value. After a 30-min preincubation, the nonvaried substrate was added to a final concentration of 20 times its K<sub>n</sub>, and the reaction rate was measured. All assays were conducted in duplicate. Glc-6-P and NAD<sup>+</sup> solutions were adjusted to pH 7.6 prior to their addition to the assay solution. Data were analyzed using double-reciprocal plots drawn with the aid of the CONC program described by Cieland (12).

Specificity Studies—Covalent modification of Glc-6-P-DH was achieved by incubating the enzyme in the dark with 422 µM [3H]PLP-AMP (1.58 × 10<sup>3</sup> cpm/mol) for various times, ranging from 1 to 45 min. After a brief incubation (approximately 15 s) with a large molar excess of sodium borohydride from a freshly prepared solution kept on ice, unbound ligand was removed from the medium by centrifugation through a 1-ml column of Sephadex G-50-80 (13) equilibrated in a solution containing 35 mM potassium phosphate, 1.0 mM EDTA, 0.2 M NaCl, pH 7.2 ("storage buffer"). The volume of the effluent was measured, and aliquots were used to determine catalytic activity, radiolabel incorporation, and protein concentration as described above.

Preparative Labeling and Peptide Isolation—Glc-6-P-DH was incubated at 9.6 µM for 10 min in storage buffer at room temperature, either without ligands or with 12 mM Glc-6-P or 72 mM NAD<sup>+</sup>; these concentrations are 10 times the respective K<sub>n</sub> values (14). [3H]PLP-AMP or [3H]PLP-AMP was added to a final concentration of 384 or 375 µM, respectively, and each sample was incubated for 10 min prior to reduction with a large molar excess of NaBH<sub>4</sub>. Unbound ligand was removed by passage through a 5-ml centrifuge column containing Sephadex G-50-80 equilibrated in storage buffer. Aliquots were withdrawn for assay of activity, protein, and bound [3H] as described above. Remaining protein was precipitated by addition of trichloroacetic acid to 14.4%, and pellets were washed with ice-cold acetone and then kept on ice, unbound ligand was removed from the medium by centrifugation through a 1-ml column of Sephadex G-50-80 that had been equilibrated with 35 mM potassium phosphate, 1.0 mM EDTA, 0.2 M NaCl, pH 7.2, to remove PLP-AMP. Fig. 1 shows that loss of activity is linearly related to the amount of PLP-AMP incorporated. Extrapolation of the plot yields a value of 0.85 mol of PLP-AMP incorporated per mol of enzyme subunit at 0% residual activity.

Inactivation by PLP-AMP and Protection by Coenzymes and Substrate—Glc-6-P-DH was incubated with [3H]PLP-AMP either in the absence of substrates, or in the presence of Glc-6-P or NAD<sup>+</sup>, each at 10 times its respective K<sub>n</sub> value (14). Following borohydride reduction and column centrifugation, enzyme activities and radiolabel incorporation were measured. Both Glc-6-P and NAD<sup>+</sup> protected against PLP-AMP inactivation and modification (Table I).

Identification of Amino Acids Modified by PLP-AMP—Labeled samples for peptide purification were prepared as described in Table I. The enzymatic activities remaining after PLP-AMP modification and the stoichiometries of PLP-AMP incorporation are shown in Table I. Proteolysis of labeled enzyme was performed with endoproteinase Lys-C, previously shown not to cleave at PLP- or PLP-AMP-modified lysyl residues (16). Equivalent weights of the initial digests were separated by HPLC, as described under "Experimental Procedures." Column eluates were monitored for absorbance at 214 (peptides), 260 (adenine, with interference from Tyr and Trp), and 325 nm (reduced pyridoxamine derivative) and for radioactivity.

RESULTS

Reversibility of Inhibition of PLP-AMP—Glc-6-P-DH was incubated at 114 µM at room temperature in 25 mM potassium phosphate, pH 7.6, with 10.2, 31.3, and 100 µM PLP-AMP. Activities were measured at various times and, by 1 h, reached equilibrium values of 82, 58, and 33%, respectively, of the original activity. The enzyme solutions were then dialyzed for 72 h against storage buffer, pH 7.2. The activities of the dialyzed enzyme solutions returned to 97, 94, and 99%, respectively, of the original activity.

Kinetics of Reversible PLP-AMP Inhibition—Inhibition of Glc-6-P-DH by PLP-AMP was measured (without borohydride reduction) using 10.5 and 31.6 µM PLP-AMP, varying either coenzyme or Glc-6-P. Inhibition was noncompetitive with respect to either NAD<sup>+</sup> or NADP<sup>+</sup> and competitive with respect to Glc-6-P using either coenzyme (data not shown). K<sub>i</sub> values for PLP-AMP were determined to be 40 µM in the NADP-linked and 34 µM in the NAD-linked reaction, respectively.

FIG. 1. Stoichiometry of PLP-AMP incorporation into Glc-6-P-DH. Glc-6-P-DH was incubated at 9.6 µM in solution containing 35 mM potassium phosphate, 1.0 mM EDTA, 0.2 M NaCl, pH 7.2, at room temperature with 422 µM [3H]PLP-AMP (1.58 × 10<sup>3</sup> cpm/mol). Aliquots were removed at various times (up to 45 min), sodium borohydride was added (60 mM final concentration), and after 15 s the sample was centrifuged through a 1-ml column of Sephadex G-50-80 that had been equilibrated with 35 mM potassium phosphate, 1.0 mM EDTA, 0.2 M NaCl, pH 7.2. The volumes of the effluents were measured and aliquots were assayed for enzyme activity, protein concentration, and radiolabel incorporation. Incorporation of PLP-AMP was calculated as moles incorporated per mol of enzyme subunit.
TABLE I
Inactivation and modification of glucose-6-phosphate dehydrogenase by [3H]PLP-AMP

| Glc-6-P-DH (9.64 μM) was incubated at room temperature for 5 min either without substrate or with 12 mM Glc-6-P or 72 mM NAD+ in 35 mM potassium phosphate, 1.0 mM EDTA, 0.2 M NaCl, pH 7.2. [3H]PLP-AMP was added to a final concentration of 38 μM and incubations continued for 10-20 min in the dark. Reduction by NaBH₄, gel filtration, and measurements of volume, enzyme activity, protein concentration, and radioisotope incorporation were performed as described in the legend to Fig. 1.

| Percent inactivation | Stoichiometry of PLP-AMP incorporation |
|----------------------|----------------------------------------|
|                      | No substrate | Glc-6-P | NAD+ | No substrate | Glc-6-P | NAD+ |
|                      |             |         |      |             |         |      |
| 59 ± 4.2             | 33 ± 4.8    | 39 ± 4.5| 0.56 ± 0.058 | 0.37 ± 0.044 | 0.37 ± 0.063 |

* Results from five independent experiments ± standard error of the mean.

In the sample labeled in the absence of protecting ligand (Fig. 2, panel A: solid bars and panel B), there were two principal peaks eluting at 22 and 29% F that accounted for the bulk of the radioactivity and the absorption at 325 nm. The amount of radioactivity eluting at fraction 11 was variable in different experiments and substrate protection was not evident. A broad band of radioactivity, mostly eluting after the two major peaks, may reflect the co-elution of the 22% F and 29% F peptides with other peptides. This was supported by the finding that when these fractions were rechromatographed, some of the label eluted at 29% F. The two principal peaks were significantly diminished in incubations that included Glc-6-P (panel A: open bars) or NAD+ (data not shown).

The peptides corresponding to the two principal peaks were collected, further purified by HPLC, and subjected to amino acid sequencing. The results are shown in Table II. The peptide isolated as the 29% F peak (Peptide 1) was labeled at Lys-21, whereas the 22% F peptide (Peptide 2) was labeled at Lys-343. Peptide isolation and sequencing were reproduced in two independent experiments.

Inactivation by PLP—Experiments were performed in which [3H]PLP incorporation was measured and peptides labeled with [3H]PLP were isolated. As found with PLP-AMP, PLP incorporation is substantially protected against inactivation by Glc-6-P and NAD+ (Table III). Samples of Glc-6-P-DH covalently modified with PLP were digested with endoproteinase Lys-C, and labeled peptides were separated by HPLC, using the same procedure as that described for PLP-AMP-labeled enzyme. Two separate experiments produced elution patterns that were indistinguishable from those seen with the peptides generated from PLP-AMP-modified Glc-6-P-DH. The peptide eluting at the same position as peptide 1 from PLP-AMP-modified enzyme was subjected to amino acid sequencing (11 cycles) and its sequence proved to be identical to that of peptide 1.

**DISCUSSION**

PLP-AMP was designed and synthesized independently in two different laboratories as an affinity label for nucleotide or coenzyme binding sites on kinases and dehydrogenases (6-8). PLP acts as an affinity label for lysyl residues that interact with phosphates, particularly sugar phosphates (4, 17). The attachment of an AMP moiety to PLP should direct this affinity label to lysyl residues of proteins, such as kinases and dehydrogenases, that have a strong affinity for adenine nucleotides. It was anticipated, therefore, that enzymes that bind nucleotides or nicotinamide coenzymes would bind PLP-AMP with greater affinity and selectivity than PLP. This is known to be the case for hexokinase, 3-phosphoglycerate kinase, adenylyl kinase, and alcohol dehydrogenase (7). Experiments described in this communication were undertaken to test this possibility with Glc-6-P-DH from *L. mesenteroides*.

The interaction of *L. mesenteroides* Glc-6-P-DH with PLP-AMP, however, appears to be identical to its interaction with PLP. Kinetic studies showed that the mechanism of reversible inhibition by PLP-AMP is competitive with respect to Glc-6-P when either NAD+ or NADP+ is the coenzyme and noncompetitive2 with respect to both NAD+ and NADP+. This is the same as the mechanism of PLP inhibition of *L. mesenteroides* Glc-6-P-DH (4). The Kᵢ values for PLP-AMP

2 Noncompetitive, rather than uncompetitive, inhibition occurs because the kinetic mechanism of the NAD-linked reaction is random, whereas it is ordered, with coenzyme binding first, for the NADP-linked reaction. The enzyme-Glc-6-P complex is utilized in the NAD-linked, but not the NADP-linked reaction (18).
PLP-AMP Modification of Glucose-6-phosphate Dehydrogenase

### Table II

**PLP-AMP-labeled peptides of glucose-6-phosphate dehydrogenase**

Peptide 1 is the "29% F" peak (Fig. 2); its amino acid sequence corresponds to residues 20-31. Peptide 2 is the "22% F" peak (Fig. 2); its amino acid sequence corresponds to residues 339-352. At the position indicated (Lys), there was a blank in the sequence; the presence of a Lys residue at this location was inferred from its presence in the complete amino acid sequence. Previous experience with radioactive phenylthiohydantoin-derivatives of PLP-Lys or PLP-AMP-Lys (16) has shown that the bulk of such derivatives do not elute from the glass-fiber disk of the gas-phase sequencer. The small amount that does elute is found superimposed with the injection artefact of the HPLC profile and is neither identifiable nor quantifiable. Peptides were sequenced in an Applied Biosystems model 475 gas-phase sequencer with on-line HPLC (Applied Biosystems model 120) phenylthiohydantoin-derivative analysis. For Peptide 1, 62.5 pmol was applied to the sequencer; the repetitive yield was 93.5%. For Peptide 2, 86 pmol was applied to the sequencer; the repetitive yield was 93%. The sequence analysis for each peptide was repeated once.

| Peptide 1 | Arg-(Lys)-Leu-Tyr-Pro-Ser-Val-Phe-Asn-Leu-Tyr-Lys |
| Peptide 2 | Arg-Leu-Ala-Ala-(Lys)-Glu-Thr-Arg-Val-Asp-Ile-Val-Phe-Lys |

### Table III

**Inactivation and modification of glucose-6-phosphate dehydrogenase by [3H]PLP**

Glc-6-P-DH (9.64 μM) was incubated at room temperature for 5 min either without substrate or with 12 mM Glc-6-P, or 70 mM NAD⁺ in 35 mM potassium phosphate, 1.0 mM EDTA, 0.2 mM NaCl, pH 7.2. [3H]PLP was added to a final concentration of 375 μM and incubations continued for 11 min in the dark. Reduction by NaBH₄, gel filtration, and measurements of volume, enzyme activity, protein concentration, and radiolabel incorporation were performed as described in the legend to Fig. 1.

| Percent inhibition* | Stoichiometry of PLP incorporation* |
|---------------------|-----------------------------------|
| No substrate Glc-6-P NAD⁺ | No substrate Glc-6-P NAD⁺ |
| 51 | 19 | 25 | 0.63 | 0.29 | 0.26 |

* Results from two independent experiments.

### Notes

- Peptides were sequenced in an Applied Biosystems model 475 gas-phase sequencer with on-line HPLC (Applied Biosystems model 120) phenylthiohydantoin-derivative analysis. For Peptide 1, 62.5 pmol was applied to the sequencer; the repetitive yield was 93.5%. For Peptide 2, 86 pmol was applied to the sequencer; the repetitive yield was 93%. The sequence analysis for each peptide was repeated once.

- Results from two independent experiments.

- Moles of PLP incorporated per mol of enzyme subunit. Results from two independent experiments.

- Percent inhibition refers to the decrease in enzyme activity compared to the control.

- Stoichiometry refers to the number of molecules of PLP incorporated per molecule of enzyme.

- Effects are virtually identical to those for PLP (4). With PLP, complete inactivation with PLP-AMP leads to the incorporation of approximately 1 mol of affinity probe/mol of subunit (4). Finally, Lys-21 is modified by both PLP and PLP-AMP. In addition, PLP is incorporated into a peptide eluting at the same position on HPLC as a peptide labeled by PLP-AMP and shown, upon sequencing, to be labeled on Lys-343. Although this PLP-labeled peptide was not sequenced, the evidence suggests that it too is modified at Lys-343. In contrast, pyridoxal and pyridoxamine 5'-phosphate are only weakly inhibitory toward L. mesenteroides Glc-6-P-DH (4). This suggests to us that the phosphate and aldehyde moieties are essential for binding or inhibition, but the results with PLP-AMP indicate that esterification of the phosphate to an AMP moiety has no perceptible influence on the binding or inhibition characteristics of the probe. It seems likely, therefore, that both PLP and PLP-AMP are functioning as affinity probes for the sugar phosphate site on L. mesenteroides Glc-6-P-DH rather than at the coenzyme site.

- Esterification of PLP-AMP by either Glc-6-P or NAD⁺, when these ligands are virtually identical to those for PLP (4). As with PLP, simultaneous binding of Glc-6-P and either probe is not possible, explaining the fact that the probes inhibit noncompetitively with respect to Glc-6-P and that Glc-6-P protects the enzyme against modification and inactivation. In contrast, NAD⁺ can still bind when PLP or PLP-AMP is bound at the Glc-6-P site, but the bound coenzyme either interferes sterically with binding of the probes or causes a conformational change that makes it difficult to form the Schiff's-base complex that is required for inactivation and modification following borohydride reduction. This interpretation is consistent with the finding that the probes inhibit noncompetitively with respect to NAD⁺, yet NAD⁺ provides some protection against modification and inactivation. Steric interference between NAD⁺ and probe is unlikely in view of the fact that NAD⁺ and Glc-6-P promote each other's binding during catalysis (11) and that covalent modification of Glc-6-P-DH with PLP increases its affinity for NAD⁺ nearly 10-fold (5). Evidence which favors interference by NAD⁺ with Schiff's-base formation includes extensive documentation for a major conformational change upon NAD⁺ binding (5, 14, 19).

- One of the lysyl residues of L. mesenteroides Glc-6-P-DH that is modified by PLP and PLP-AMP, Lys-21, is part of a highly conserved region of 14 amino acids in Glc-6-P-DHs from human erythrocytes (20, 21), rat liver (22, 23), opossum (24), Drosophila melanogaster (25), and L. mesenteroides (26). Lys-343 corresponds to an arginyl residue in the Glc-6-P-DHs from human erythrocytes, rat liver, and Drosophila (20-23, 25), with a lysyl residue adjacent to it. This region of the L. mesenteroides Glc-6-P-DH is not as homologous with the other Glc-6-P-DHs as is the region containing Lys-21.

- Previously, a pyridoxyllysine-containing peptide from PLP-labeled L. mesenteroides Glc-6-P-DH was isolated and sequenced before the entire amino acid sequence of the enzyme was known (3). Nonradioactive PLP was used in that study and the modified peptide was identified from its absorbance at 315 nm and its fluorescence at 390 nm upon excitation at 325 nm. The sequence of the PLP-labeled peptide determined earlier (3) does not correspond to any portion of the recently determined L. mesenteroides Glc-6-P-DH sequence (3). We are unable to provide a satisfactory explanation of this finding but assume that a contaminating peptide was co-purified, obscuring the previous sequence analysis.

- Human erythrocyte Glc-6-P-DH is inhibited by PLP, and Glc-6-P, but not NAD⁺, protects effectively against this inhibition. This difference offers the opportunity to address the question of which portion of the enzyme is modified by PLP, and at what point the modified site becomes inactivated. Previous experience with radioactive phenylthiohydantoin-derivatives of PLP-Lys or PLP-AMP-Lys (16) has shown that the bulk of such derivatives do not elute from the glass-fiber disk of the gas-phase sequencer. The small amount that does elute is found superimposed with the injection artefact of the HPLC profile and is neither identifiable nor quantifiable. Peptides were sequenced in an Applied Biosystems model 475 gas-phase sequencer with on-line HPLC (Applied Biosystems model 120) phenylthiohydantoin-derivative analysis. For Peptide 1, 62.5 pmol was applied to the sequencer; the repetitive yield was 93.5%. For Peptide 2, 86 pmol was applied to the sequencer; the repetitive yield was 93%. The sequence analysis for each peptide was repeated once.

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inhibition (26). The lysyl residue modified is part of a sequence of 13 amino acids that is identical in Glc-6-P-DHs from human erythrocytes, L. mesenteroides (27, 28), rat liver (20, 23), and Drosophila (25). Homologous peptides consisting of 11 amino acids have also been isolated from Glc-6-P-DHs from Pichia jadinii (29) and Saccharomyces cerevisiae (30). In the Glc-6-P-DHs from yeast (31) and P. jadinii (29) the lysyl residue in this conserved sequence is selectively modified by acetylsalicylic acid. Again, Glc-6-P, but not NADP+, protects effectively against modification (31). This conserved lysine-containing sequence is, therefore, likely to form part of the active site, and the evidence with the Glc-6-P-DHs from human erythrocytes, S. cerevisiae, and P. jadinii suggests that it may be involved in Glc-6-P binding. This conserved sequence includes Lys-182 in L. mesenteroides Glc-6-P-DH, and our results show that this lysyl residue is not covalently modified with PLP-AMP or PLP. L. mesenteroides Glc-6-P-DH is unique, among the Glc-6-P-DHs for which amino acid sequence information is available, in its ability to utilize either NAD+ or NADP+ as its coenzyme (1, 2), and this is expected to lead to some differences in its interactions with Glc-6-P and/or coenzymes. Its interactions with NAD+ and Glc-6-P has been shown to involve major conformational changes (5, 14, 19). One or both of the lysyl residues covalently modified by PLP and PLP-AMP may be located in regions of the enzyme undergoing these ligand-induced conformational changes. It has been shown recently that PLP-AMP-labeled lysyl residues in hexokinase (32) and 3-phosphoglycerate kinase (33) must traverse considerable distances during the ligand-induced conformational closure of the central binding cleft on these enzymes. Support for similar conformational changes involving those regions of L. mesenteroides Glc-6-P-DH containing Lys-21 and/or Lys-345 must await the results of detailed x-ray structural studies of this enzyme, currently in progress (34).

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