D-3-Phosphoglycerate Dehydrogenase from Mycobacterium tuberculosis Is a Link between the Escherichia coli and Mammalian Enzymes*

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Sanghamitra Dey‡, Zhiqin Hu§, Xiao Lan Xu§, James C. Sacchettini‡, and Gregory A. Grant§¶

From the Departments of Molecular Biology & Pharmacology and Medicine, Washington University School of Medicine, St. Louis, Missouri 63110 and the Department of Biochemistry & Biophysics, Texas A&M University, College Station, Texas 77843

D-3-Phosphoglycerate dehydrogenase (PGDH) from Mycobacterium tuberculosis has been isolated to homogeneity and displays an unusual relationship to the Escherichia coli and mammalian enzymes. In almost all aspects investigated, the M. tuberculosis enzyme shares the characteristics of the mammalian PGDHs. These include an extended C-terminal motif, substrate inhibition kinetics, dependence of activity levels and stability on ionic strength, and the inability to utilize α-ketoglutarate as a substrate. The unique property that the M. tuberculosis enzyme shares with E. coli PGDH that it is very sensitive to inhibition by L-serine, with an IC₅₀ = 30 μM. The mammalian enzymes are not inhibited by L-serine. In addition, the cooperativity of serine inhibition appears to be modulated by chloride ion, becoming positively cooperative in its presence. This is modulated by the gain of cooperativity in serine binding for the first two effector sites. The basis for the chloride modulation of cooperativity is not known, but the sensitivity to serine inhibition can be explained in terms of certain amino acid residues in critical areas of the structures. The differential sensitivity to serine inhibition by M. tuberculosis and human PGDH may open up interesting possibilities in the treatment of multidrug-resistant tuberculosis.

In some organisms, such as Escherichia coli (7), PGDH is strongly inhibited by l-serine (IC₅₀ = ~2–4 μM), the end product of the pathway. E. coli PGDH is the most studied and is classified as a V-type enzyme (8) because l-serine regulates catalysis by altering the velocity of the reaction rather than the affinity of the substrate. In Bacillus subtilis, inhibition of PGDH by l-serine is less sensitive (IC₅₀ = 0.6 mM) but appears to lose its sensitivity to l-serine under oxidizing conditions (9). PGDH from Corynebacterium glutamicum has also been reported to be inhibited by l-serine only at very high concentrations (IC₅₀ = 10 mM) but has not been studied in homogeneous form (10). In addition, l-serine inhibition of both the B. subtilis and C. glutamicum PGDH require extensive preincubation of the enzyme with the inhibitor before appreciable inhibition can be measured. In the pea (Pisum sativum), the sensitivity to l-serine has been reported to be cold labile (3). In crude extracts of wheat germ (4), PGDH activity appears to be inhibited by high concentrations of l-serine, but this sensitivity is reported to be lost upon incubation, and the purified wheat germ PGDH is not inhibited by L-serine. L-Serine has been reported to have no effect on the activity of PGDH from rat liver (11) and chicken liver (12). The effect of L-serine on the activity of PGDH from other species has not been reported.

PGDH exists in at least three different basic structural motifs that do not appear to be strictly specific for organism type (11). The PGDH of some bacteria and some lower eukaryotes, such as yeast, Leishmania, and Neurospora, are structurally similar to the E. coli enzyme (Figs. 1 and 2). In addition to substrate and nucleotide-binding domains, they possess a homologous C-terminal domain that, in E. coli, is involved in effector binding (l-serine) and regulation of activity. This third domain has been called the “ACT domain” and consists of a βαβαβ structural motif that has been found in other proteins as well (13, 14). Other bacteria and higher order eukaryotes, including mammals, possess a large polypeptide insertion in their C-terminal segment immediately following the substrate-binding domain. Furthermore, the homology to the ACT domain following this insertion is minimal. A third motif, which lacks the C-terminal regulatory domain altogether, is also found in some bacteria, including some, such as Mycobacterium tuberculosis, that also produce PGDH with the extended C-terminal motif. Recently, a variation of this third motif has been recognized in the parasite Entamoeba histolytica (15) that appears to utilize a lysyl residue rather than a histidyl residue at the active site for proton transfer.

In general, PGDH enzymes with a structural motif similar to that of E. coli possess the requisite serine-binding residues in the regulatory domain. However, all of these enzymes except E. coli PGDH appear to be missing a critical hinge region.

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‡To whom correspondence should be addressed: Dept. of Molecular Biology and Pharmacology, Box 8103, Washington University School of Medicine, 660 S. Euclid Ave., St. Louis, MO 63110; Tel.: 314-362-3367; Fax: 314-362-4698; E-mail: ggrant@molecool.wustl.edu.

§ The abbreviations used are: PGDH, D-3-phosphoglycerate dehydrogenase; HPAP, hydroxypropyruvic acid phosphate.
The polypeptide arrangement of PGDH falls into four general categories. Top bar, PGDH from some bacteria and simple eukaryotes consist of three distinct domains called the cofactor or nucleotide-binding domain, which also contains the active site histidine (H), the substrate-binding domain, and the regulatory or serine binding domain. The substrate-binding domain is a distinct structural entity formed by two polypeptide segments that flank the nucleotide-binding domain in the sequence. The regulatory domain has also been called the ACT domain. These proteins form a second domain interface at the regulatory domain and exist as tetramers. They also contain an invariant tryptophan (Trp) that inserts into a pocket on the adjacent subunit at the nucleotide-binding domain interface. Second bar, PGDH from other bacteria and higher order prokaryotes such as mammals contain a large insert between their substrate-binding domain segment and the segment displaying homology with the regulatory domain. These proteins also form a second domain interface at the regulatory domain and exist as tetramers, and they also contain the invariant tryptophan. Third bar, some organisms also contain a PGDH that is devoid of a regulatory domain segment. They exist as dimers and may or may not retain Trp. These organisms appear to always contain PGDH with one of the other motifs. Bottom bar, a few organisms have been found to contain a PGDH where the active site histidine (H) has been replaced by a lysine residue (K). They are also dimers and are missing Trp. The symbol ~ indicates that the N termini are of various lengths (see Fig. 2).

Consisting of two adjacent glycine residues that appears to be critical for the ability of serine to inhibit the active site (16). The PGDH from rat, with the extended C-terminal motif similar to that found in other mammals, has been reported to be insensitive to L-serine. Other mammalian enzymes such as those from the mouse and human have not been characterized, but the human enzyme exhibits 94% amino acid identity with the rat enzyme.

PGDH from M. tuberculosis contains an extended C-terminal domain like that found in mammalian PGDH, but we have observed that it is readily inhibited by L-serine. In fact, it is the only other purified PGDH that has been shown to be inhibited by L-serine. Although it has not yet been characterized, the human enzyme is expected to have properties similar to the rat PGDH, which is insensitive to L-serine, because they are 94% identical.

Tuberculosis is a deadly disease that is prevalent throughout much of the world and that is showing significant development of multidrug resistance. If the human enzyme is, in fact, insensitive to L-serine, this may provide a possible avenue toward the development of new drugs to treat tuberculosis. This study reports the expression and characterization of L-serine-sensitive PGDH from M. tuberculosis and demonstrates that it possesses a unique mode of regulation.

MATERIALS AND METHODS

PGDH activity was measured by following the decrease in absorbance at 340 nm in the presence of NADH and hydroxypropionic acid phosphate (HPAP). Unless otherwise indicated, the assays were performed in 200 mM potassium phosphate buffer, pH 7.5, containing 1 mM isopropyl-β-D-thiogalactopyranoside and allowed to grow at 37 °C for 6–7 h. The cells are harvested by centrifugation at 2,000 × g for 2 h, and the supernatant is dialyzed against 20 mM potassium phosphate, pH 7.5, 5 mM KCl. After the addition of lysozyme (0.16 mg/ml), the cells are disrupted by sonication for 2–3 min. Cell debris is removed by centrifugation at 27,000 × g for 1 h, and the supernatant is dialyzed against 20 mM potassium phosphate, pH 7.5, 5 mM KCl in preparation for ion exchange chromatography. A 2.6 × 8-cm column of Q-Sepharose is equilibrated in 20 mM potassium phosphate, pH 7.5, 5 mM KCl. After the sample is applied to the column, the column is washed with starting buffer until the A280 reaches approximately 0. The protein is eluted with a 120-ml linear gradient of 25 to 1000 mM KCl in 20 mM potassium phosphate, pH 7.5, and the fractions are collected. M. tuberculosis PGDH is located by assaying for enzymatic activity, and the appropriate fractions are pooled. The pooled fractions are dialyzed against 100 mM potassium phosphate, pH 7.5, 5 mM KCl and chromatographed on an identical Q-Sepharose column with an linear gradient from 25 to 1000 mM KCl.

Sequence Homology—All of the sequences were obtained from the National Center for Biotechnology Information ENTREZ data base (www.ncbi.nlm.nih.gov/). Sequence alignments were initially performed using the SIM program from the ExPASy Molecular Biology server at expasy.org/tools/.

Serine Inhibition and Binding—Serine inhibition plots were fit to

![Diagram](image-url)
FIG. 2. Sequence homology of D-3-phosphoglycerate dehydrogenase. The PGDH sequences shown are from *E. coli* (**ec**, accession number AAC75950), *Leishmania major* (**le**, accession number NP_859431), *Saccharomyces cerevisiae* (**ye**, accession number P40054), *M. tuberculosis* (**tb**, accession number 53243), *C. glutamicum* (**cg**, accession number NP_600506), *B. subtilis* (**bs**, accession number P35136), *Rattus norvegicus* (**rl**, accession number CAB 89828), and *Homo sapiens* (**hu**, accession number AAH11262). The figure is numbered in reference to positions within the figure and not with reference to the residue number of any specific enzyme. The yeast enzyme contains a long N-terminal extension that is not numbered. Dashes were inserted to optimize homology. Residues that are identical in all sequences are designated with an asterisk. Residues involved in serine binding in *E. coli* PGDH are designated with a plus sign. Functional regions are: positions 156–164 and 182, nucleotide binding; position 243, arginine that forms ionic interaction with the C-1 carboxyl of the substrate; position 272, glutamic acid that is part of the Glu-His catalytic dyad; positions 302–312, conserved area around the active site histidine (position 303); and positions 493, 495, and 513, histidine and asparagine ligands for serine binding. In addition, positions 347–348 (marked with plus sign) are the Gly-Gly residues in *E. coli* PGDH that are a putative hinge region.
the Hill equation as previously described (17). Binding of L-serine to *M. tuberculosis* PGDH was performed as described previously (18) using tritiated L-serine. Stepwise intrinsic dissociation constants for L-serine were determined by fitting to the Adair equation for four binding sites as previously described. Hill plots were utilized to determine the dissociation constant for the binding of the first serine (19). This was subsequently used to constrain $K_1$ in the Adair equation. All of the data were fit with Kaleidograph version 3.6 from Synergy Software.

**RESULTS**

*M. tuberculosis* is a prokaryote, but the amino acid sequence of its PGDH more closely resembles that from mammalian species (Fig. 2). PGDH from *M. tuberculosis* has been isolated to homogeneity and characterized. It runs as a single band on SDS-PAGE with a subunit molecular weight of 55,000 that is consistent with the molecular weight predicted from its sequence (Fig. 3).

**Activity, Substrate Specificity, and Stability**—The physiological substrate of PGDH from all species is hydroxypyruvic acid phosphate. *E. coli* PGDH can also utilize $\alpha$-ketoglutarate as a substrate (20). Neither substrate produces inhibition of *E. coli* PGDH activity at high concentrations. In contrast, *M. tuberculosis* PGDH is specific for hydroxypyruvic acid phosphate as a substrate as was also demonstrated for rat liver PGDH. Neither enzyme displays any activity with $\alpha$-ketoglutarate. In addition, *M. tuberculosis* PGDH exhibits substrate inhibition (Fig. 4) similar to what has been reported for rat liver PGDH (11). Utilizing the initial part of the curve in a $v$ versus $S$ plot and fitting it to the Michaelis-Menten equation, the $K_m$ for hydroxypyruvic acid phosphate is $85 \mu M$, and the $k_{cat}/K_m$ is $-5.6 \times 10^4 \text{ mol}^{-1} \text{ s}^{-1}$. The *E. coli* enzyme has a $K_m$ of $-40 \mu M$ and a $k_{cat}/K_m$ of $-7 \times 10^4 \text{ mol}^{-1} \text{ s}^{-1}$ for hydroxypyruvic acid phosphate. For $\alpha$-ketoglutarate the *E. coli* enzyme has a $K_m$ of $0.5 \text{ mM}$ and a $k_{cat}/K_m$ of $-3 \times 10^4 \text{ mol}^{-1} \text{ s}^{-1}$. The pH at which *M. tuberculosis* PGDH displays optimal activity is 6.5, whereas that for *E. coli* PGDH is 8.5. Although *M. tuberculosis* PGDH utilizes NADH to catalyze the same reaction as the *E. coli* and rat liver enzymes, it is not retained on a column of 5'AMP-Sepharose as they are. These characteristics are summarized in Table I.

As has been reported for rat liver PGDH (11), the ionic strength of the buffer affects the substrate inhibition characteristics of *M. tuberculosis* PGDH (Fig. 5). Optimal activity is seen at $-75$ to $100 \text{ mM}$ potassium phosphate. Only about 30% of the optimal activity can be measured in 5 mM potassium phosphate. Increasing ionic strength also reduces the degree of substrate inhibition and shifts the maximal activity to higher substrate concentrations (Fig. 4).

Ionic strength is also critical for the stability of the purified enzyme. At low ionic strength the enzyme irreversibly loses activity with time (Fig. 6). For example, in 20 mM phosphate buffer, pH 7.5, most of the activity is lost within 24 h. The activity loss is prevented if the ionic strength is kept above $-100 \text{ mM}$ salt. It is not specific for phosphate buffer because the...
same protection is gained with salts such as KCl and NaCl.

Furthermore, the rate of activity loss is dependent on the concentration of the enzyme (Fig. 7). At low ionic strength, the more dilute the enzyme, the faster the rate of loss of activity. However, dilute enzyme can be protected from the loss of activity by a higher ionic strength environment. The activity loss profiles in Fig. 7 could only be fit well with an equation representing biphasic exponential decay, suggesting that the loss of activity proceeds by a two-step mechanism. None of these effects of ionic strength are observed with \textit{E. coli} PGDH.

**Inhibition by L-Serine—** Rat liver PGDH contains an extended C-terminal motif and is reported to be insensitive to L-serine. In contrast, \textit{M. tuberculosis} PGDH, which also contains the extended C-terminal motif, is easily inhibited by L-serine, with an \( I_{0.5} \) for L-serine of \(-30 \mu M\). This compares to \(-2–4 \mu M\) for \textit{E. coli} PGDH. \textit{E. coli} PGDH also shows positive cooperativity for serine inhibition with a Hill coefficient of \(-2.0\). Unlike the \textit{E. coli} enzyme, when assayed in phosphate buffer, \textit{M. tuberculosis} PGDH displays a Hill coefficient of \(-1.0\), indicating the absence of cooperativity for serine inhibition. However, positive cooperativity can be induced by the presence of chloride ions. Table II demonstrates that when chloride ion is present in the phosphate buffer, Hill coefficients greater than 1.0 are observed. Although the effect of chloride on the Hill coefficient seems to be optimal at high chloride ion concentrations (250 mM), the effect is present at physiological levels of chloride ion as well (0.02–0.10 mM). This effect does

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**Table I**

Properties of \textit{E. coli}, \textit{M. tuberculosis}, and rat liver \( \delta \)-3-phosphoglycerate dehydrogenases

|                      | \textit{E. coli}\textsuperscript{a} | \textit{M. tuberculosis}\textsuperscript{b} | Rat liver\textsuperscript{c} |
|----------------------|-----------------------------------|---------------------------------|-----------------|
| Subunit molecular weight | 44,044                            | 54,522                          | 56,650          |
| pH optimum           | 8.5                               | 6.5                             | NR\textsuperscript{d} |
| Substrates           | HPAP and aKG\textsuperscript{e}    | HPAP                             | HPAP            |
| Sensitive to ionic strength | No                               | Yes                             | Yes             |
| Affinity for 5' AMP-Sepharose | Yes                              | No                              | Yes             |
| Inhibited by L-serine | Yes                               | Yes                             | No              |

\textsuperscript{a} From this study and Refs. 16–19 and 27.  
\textsuperscript{b} From this study.  
\textsuperscript{c} See Ref. 11.  
\textsuperscript{d} Not reported. Assays were performed at pH 7.1.  
\textsuperscript{e} aKG, α-ketoglutarate.

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**Fig. 4.** Substrate inhibition in \textit{M. tuberculosis} PGDH. A plot of substrate concentration versus level of catalytic activity (units/ml) display substrate inhibition at high concentrations of phosphohydroxy-pyruvic acid (\([S]\)). The degree of inhibition and concentration for optimal activity varies as a function of the ionic strength. ●, 20 mM KPO\textsubscript{4}, pH 7.5; ■, 200 mM KPO\textsubscript{4}, pH 7.5.

**Fig. 5.** Dependence of \textit{M. tuberculosis} PGDH catalytic activity on ionic strength. The activity (units/ml) of \textit{M. tuberculosis} PGDH is shown assayed at various concentrations of phosphate buffers at pH 7.5. Equal amounts of enzyme (\(-0.35 \mu M\)) are used in each assay.

**Fig. 6.** Effect of ionic strength on the stability of \textit{M. tuberculosis} PGDH. Freshly prepared \textit{M. tuberculosis} PGDH was dialyzed against the buffers indicated and assayed immediately after dialysis (gray bars) and 24 h later (black bars). Activity is in units/ml.
not involve the potassium ion because it is present in the phosphate buffer salts, and NaCl produces similar results. When chloride ion is missing, even at high salt concentrations such as 250 mM sodium acetate, the effect is not observed. The $I_{0.5}$, the concentration of inhibitor at which half-maximal inhibition is observed, also increases with the chloride ion concentration. So, although chloride induces cooperativity, it decreases sensitivity to serine. Table II also demonstrates that the effect is maximal for chloride ion among the halogen group. Fluoride ion does not induce cooperativity, whereas bromide and iodide display a small effect. In addition, tartrate ions, which were identified in the crystal structure at an anionic binding site between the intervening and regulatory domains, do not induce cooperativity (Table II).

Double reciprocal plots of velocity versus substrate concentration (Fig. 8) display the upward curvature at high substrate concentrations expected for substrate inhibition. Extrapolation of the data at low substrate concentration show that inhibition by L-serine is not of the competitive type and is most likely of the mixed noncompetitive type. The E. coli enzyme displays strict noncompetitive inhibition with HPAP. This indicates that L-serine most likely binds at an allosteric site somewhere in the C-terminal domain similar to that of the E. coli enzyme.

In the accompanying paper (21) that describes the crystal structure of M. tuberculosis PGDH as a function of concentration, time, and ionic strength. The enzyme was diluted at zero time from 200 mM potassium phosphate buffer, pH 7.5, and assayed as a function of time. ●, 1/100 dilution in 200 mM potassium phosphate buffer, pH 7.5; ■, 1/500 dilution in 200 mM potassium phosphate buffer, pH 7.5; ●, 1/10 dilution in 20 mM potassium phosphate buffer, pH 7.5; ▲, 1/100 dilution in 20 mM potassium phosphate buffer, pH 7.5; ▼, 1/500 dilution in 20 mM potassium phosphate buffer, pH 7.5.

The amount of enzyme present in the assay cuvette was kept constant (0.35 M). The assays were conducted in 200 mM potassium phosphate buffer, pH 7.5. Activity is in units/ml.

**Table II**

| Concentration | Salt    | Hill coefficient $I_{0.5}$ | $\mu$M  |
|---------------|---------|---------------------------|--------|
| None         | none    | 0.98 ± 0.09               | 21.2 ± 1.8 |
| 0.02 mM KCl  | KCl     | 1.38 ± 0.07               | 18.2 ± 0.8 |
| 0.10 mM KCl  | KCl     | 1.41 ± 0.08               | 26.1 ± 1.0 |
| 50 mM KCl    | KCl     | 1.45 ± 0.09               | 29.9 ± 1.4 |
| 50 mM NaCl   | NaCl    | 1.59 ± 0.07               | 30.8 ± 0.9 |
| 150 mM KCl   | KCl     | 1.38 ± 0.10               | 63.3 ± 3.0 |
| 250 mM KCl   | KCl     | 1.65 ± 0.08               | 123.3 ± 3.2 |
| 250 mM Sodium acetate | KCl | 1.06 ± 0.08               | 17.7 ± 1.3 |
| 50 mM NaF    | NaF     | 1.05 ± 0.05               | 38.1 ± 1.5 |
| 50 mM NaBr   | NaBr    | 1.21 ± 0.06               | 66.8 ± 2.2 |
| 50 mM NaI    | NaI     | 1.14 ± 0.08               | 78.2 ± 3.9 |
| 10 mM Potassium tartrate | | 1.17 ± 0.07               | 27.6 ± 0.1 |

*All conditions are in 200 mM potassium phosphate buffer, pH 7.5.*
structure of \(M. \text{tuberculosis}\) PGDH, the serine-binding site has been tentatively identified by structural and sequence homology to the serine site in \(E. \text{coli}\) PGDH. However, the spatial relationship of the effector site to the active site is altered by the presence of an intervening domain.

**L-Serine Binding**—The binding of L-serine to \(M. \text{tuberculosis}\) PGDH as a function of serine concentration in the absence and presence of KCl is shown in Figs. 9 and 10, respectively. The stepwise intrinsic dissociation constants derived from the data are listed in Table III. Fitting of the data with the Adair equation with four variables produced values with relatively large error values such that it was difficult to interpret the results. Fitting the data with a Hill plot (shown in the insets to Figs. 9 and 10) allowed the value for \(K_1^*\) to be derived independently. Constraining the Adair equation using this value reduced the error values for the other dissociation constants so that a more meaningful evaluation could be made. The results show that in the absence of KCl, there appears to be little cooperativity in the binding of the first two serines. The third serine appears to bind with increased affinity, whereas the final serine shows very little affinity for the protein. A similar pattern is seen in the presence of KCl except that now the binding of the first two serines exhibit positive cooperativity as well as the third. The relationship between ligand binding data and the inhibition data can be explained on the basis of the half-the-sites reactivity determined for L-serine binding to \(E. \text{coli}\) PGDH (17). In \(E. \text{coli}\) PGDH the binding of the first two serine molecules are responsible for inhibition of the active site. Binding of more than two serines may occur, but they have no additional effect on the inhibition of activity. If a similar mechanism is functional in \(M. \text{tuberculosis}\) PGDH, the dissociation constants of only the first two serines to bind should be considered within the context of inhibition of activity. Thus, in the presence of KCl, the binding and the inhibition are cooperative, and in the absence of KCl they are not.

**DISCUSSION**

Although \(\beta\)-3-phosphoglycerate dehydrogenases from \(E. \text{coli}\) and \(M. \text{tuberculosis}\) catalyze identical reactions in the first committed step for the biosynthesis of L-serine, they exhibit markedly different characteristics, and \(M. \text{tuberculosis}\) PGDH shares many characteristics with rat liver PGDH. These include the extended C-terminal motif, substrate inhibition kinetics, dependence of activity levels and stability on ionic strength, and the inability to utilize \(\alpha\)-ketoglutarate as a substrate. However, the \(M. \text{tuberculosis}\) enzyme does not display the affinity for 5'-AMP-Sepharose that the rat liver and \(E. \text{coli}\) enzyme do, although all three utilize NAD as a cofactor. A possible reason for this is addressed in the accompanying article (21), which describes the crystal structure of \(M. \text{tuberculosis}\) PGDH.

The single property that \(M. \text{tuberculosis}\) PGDH shares with the \(E. \text{coli}\) enzyme, other than utilizing their common physiological substrates, is that it is inhibited by L-serine at relatively low levels. Interestingly, in potassium phosphate buffer alone, \(M. \text{tuberculosis}\) PGDH is not inhibited in a positively cooperative manner by L-serine, as is \(E. \text{coli}\) PGDH. However, the presence of chloride ion converts the inhibition by L-serine from a noncooperative to a cooperative process. Chloride ion is not a requirement for the positively cooperative inhibition of \(E. \text{coli}\) PGDH by L-serine. Moreover, this modulation occurs at relatively low levels of chloride ion, certainly within physiological levels. As the chloride ion is increased, the \(I_{0.5}\) for L-serine increases, but the value for the Hill coefficient remains >1. Thus, the effect of chloride seems to be fully in force at a relatively low level. The effect of the chloride ion is also manifest in the binding of L-serine to the enzyme, which shows positive cooperativity in the binding of the first two serine molecules in the presence of chloride ion but not in the absence. This is consistent with the mechanism of serine inhibition in \(E. \text{coli}\), where it was shown that inhibition of activity is dependent on only the first two serines to bind. It is not known at this point how the chloride ion contributes to this phenomenon.

Modulation of enzyme activity by chloride ion has been reported in a number of cases. For example, it has been reported that rat liver glucose-6-phosphatase (22) and glycogen synthase (23) are inhibited by chloride ion. On the other hand, human cathepsin C (24) is activated by chloride ion. Chloride ion also produces a minor heterotropic effect on hemoglobin, but it does not alter the hill coefficient for ligand binding (25). Thus, the observation that the cooperativity of inhibition is modulated specifically by chloride ion appears to be relatively

**TABLE III**

Dissociation constants for L-serine binding to \(M. \text{tuberculosis}\) PGDH

| \(K_i^*\) | –KCl | +KCl |
|---------|-----|-----|
| \(K_1^*\) | \(53.7 \pm 21.4\) | \(63\) |
| \(K_2\) | \(58.7 \pm 41.8\) | \(53.4 \pm 14.0\) |
| \(K_3\) | \(16.1 \pm 6.8\) | \(18.2 \pm 5.2\) |
| \(K_4\) | VL* | VL |
| \(R^2\) | 0.9773 | 0.9906 |

* VL, very large.
unique. The crystal structure of M. tuberculosis PGDH does not reveal the site for chloride binding. A potential site for the binding of anionic molecules has been identified by virtue of the binding of tartrate molecules from the crystallization buffer at a site between the intervening and regulatory domains (21).

However, tartrate itself does not exhibit an effect on the cooperativity of inhibition by serine.

Fig. 2 presents a sequence alignment of PGDH from eight species. Four are from prokaryotic sources, and four are from eukaryotic sources, two of which are mammalian. Five of these species contain the large C-terminal extension, but only two are eukaryotic organisms. The two other eukaryotes are missing the C-terminal extension, and three prokaryotes contain it. There are 53 residues or 15% that are identical in the N-terminal portion of all the species shown (up to position 346 in Fig. 2). These include residues known to be involved in cofactor binding and at the active site. After position 346, there is less than 1% identity in all eight sequences and less than 2% identity in the C-terminal extensions found in five of the sequences.

Of the PGDH enzymes reported to be inhibited by l-serine, their sensitivity to serine ranges over 3 orders of magnitude. The concentration of l-serine that produces half-maximal inhibition of catalytic activity for PGDH from E. coli, M. tuberculosis, C. glutamicum, and B. subtilis are ~3, 30, 640, and 10,000 μM. Some insight into the structural basis for serine sensitivity among these species may be gained from inspection of the homology based on what is known about the E. coli enzyme, although it is not entirely straightforward. In E. coli PGDH, l-serine binds to the allosteric site through hydrogen bonding interaction with a histidine and two asparagine residues. These are found at positions 493, 495, and 513 in Fig. 2. In addition, a potential molecular hinge, defined by a Gly-Gly sequence at positions 356 and 357 in E. coli PGDH, seems to be required for optimal serine inhibition. It has been shown that the Gly-Gly sequence can be replaced by an Ala-Ala sequence without a major effect (16). However, residues with larger side chains interfere significantly with serine-induced inhibition. PGDH from Leishmania and yeast possess the requisite serine-binding residues but are missing the Gly-Gly hinge region. Although not yet tested, they would be predicted to be insensitive to l-serine. On the other hand, PGDH from M. tuberculosis and C. glutamicum possess a Gly-Gly sequence in this area, but their serine-binding residues differ at two positions. However, these residues are capable of forming hydrogen bonds and may function similarly. Both mammalian enzymes are completely devoid of any of these necessary features. The rat liver PGDH has been shown to be completely insensitive to l-serine. A similar situation would be predicted for the human enzyme because it is 94% identical. However, several things are left unexplained by this simple analysis. The C. glutamicum and M. tuberculosis enzymes both have the same residues corresponding to the serine binding positions as well as a Gly-Gly sequence in the right general area, but the C. glutamicum enzyme has been reported to be over a 100-fold less sensitive to l-serine. On the other hand, the B. subtilis enzyme, which has very similar serine-binding residues (but with an Asp replacing an Asn), does not contain a recognizable hinge in the region of position 356. When comparing the enzymes with the C-terminal extension to that of E. coli, the effect of the additional polypeptide must be taken into account and may change the manner in which serine interacts and inhibits the enzyme quite significantly.

The structural and kinetic characteristics of the M. tuberculosis enzyme indicate that it is more closely related to the mammalian PGDHs. However, its sensitivity to serine inhibition suggests that it possesses metabolic features more consistent with the E. coli enzyme. These observations suggest that the M. tuberculosis PGDH may be a link between the E. coli and mammalian enzymes. This hypothesis is supported by the phylogenetic tree presented by Ali et al. (15).

The intracellular soluble levels of l-serine in E. coli and B. subtilis have been reported to be in the 0.5–1.5 mM range (9). If a similar l-serine concentration is found in M. tuberculosis, the IC50 for l-serine for M. tuberculosis PGDH suggests that it may well be regulated in vivo by l-serine pools. L-Serine is a central cellular intermediate in bacteria that serves as a source for the synthesis of protein, glycine, cysteine, tryptophan, phospholipids, purines, and single carbon metabolites. In fact, ~15% of glycolytic flux is directed into the l-serine biosynthetic pathway (26). In E. coli, PGDH is a constitutive enzyme; that is it cannot grow without an external source of l-serine (27). The gene that codes for M. tuberculosis PGDH, SerA1, has been shown to be an essential gene in that organism (28). Thus, the possibility exists that analogs of l-serine that cannot be used otherwise by the M. tuberculosis organism could be potential new drugs for the control of active M. tuberculosis infection when it is growing mainly on carbon as a source.

The mechanism of E. coli PGDH has been studied in great detail. A recent report has shown that the enzyme displays half-of-the-sites activity for both catalysis and regulation by l-serine (17). Furthermore, that investigation has shown that the magnitude of inhibition of activity depends on the intersubunit relationship of effector site to active site and that this relationship can begin to be defined in a quantitative manner. This picture of how the various ligand sites in PGDH interact has more clearly defined the intermolecular mechanism of allosteric regulation in this enzyme and presented the question as to how general the mechanism may be, not only across species within homologous proteins, but as a general regulatory mechanism for enzymes of diverse function that contain the ACT domain. The unique properties of M. tuberculosis PGDH provide an intriguing and potentially enlightening opportunity to explore this question in regard to the former case and will contribute to our understanding of protein regulatory mechanisms.

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