Phosphoglycerate dehydrogenases exist in at least three different structural motifs. The first d-3-phosphoglycerate dehydrogenase structure to be determined was from *Escherichia coli* and is a tetramer composed of identical subunits that contain three discernable structural domains. The crystal structure of d-3-phosphoglycerate dehydrogenase from *Mycobacterium tuberculosis* has been determined at 2.3 Å. This enzyme represents a second structural motif of the d-3-phosphoglycerate dehydrogenase family, one that contains an extended C-terminal region. This structure is also a tetramer of identical subunits, and the extended motif of 135 amino acids exists as a fourth structural domain. This intervening domain exerts quite a surprising characteristic to the structure by introducing significant asymmetry in the tetramer. The asymmetric unit is composed of two identical subunits that exist in two different conformations characterized by rotation of ~180° around a hinge connecting two of the four domains. This asymmetric arrangement results in the formation of two different and distinct domain interfaces between identical domains in the asymmetric unit. As a result, the surface of the intervening domain that is exposed to solvent in one subunit is turned inward in the other subunit toward the center of the structure where it makes contact with other structural elements. Significant asymmetry is also seen at the subunit level where different conformations exist at the NAD-binding site and the putative serine-binding site in the two unique subunits.

**L-Serine acts as a key intermediate in a number of important metabolic pathways. It can be synthesized from the glycolytic intermediate, d-3-phosphoglycerate, in the phosphorylated pathway of serine biosynthesis. d-3-Phosphoglycerate dehydrogenase (PGDH, EC 1.1.1.95)** catalyzes the first committed step in this conversion of d-3-phosphoglycerate to phosphohydroxypyruvate utilizing NAD⁺ as a cofactor. Subsequently, phosphohydroxypyruvate is converted to phosphoserine by phosphoserine transaminase and then to l-serine by phosphoserine phosphatase (1, 2). PGDH also belongs to a family of proteins classified as 2-hydroxy acid dehydrogenases that are generally specific for substrates with a D-configuration (3). It shares sequence homology with other members of this family such as formate dehydrogenase and glycerate dehydrogenase whose structures are also known (4, 5).

PGDH appears to be ubiquitously found in all organisms. It exists in at least three different basic structural motifs that do not appear to be strictly specific for organism type (6). The PGDH of some bacteria and some lower eukaryotes, such as yeast and Neurospora are similar to the *Escherichia coli* enzyme. In addition to substrate and nucleotide-binding domains, they possess a homologous C-terminal domain that, in *E. coli*, is involved in regulation of activity through binding the effector, l-serine. Other bacteria such as *Mycobacterium, Bacillus subtilis, Corynebacterium*, plants such as Arabidopsis, and higher order eukaryotes, including mammals, possess a large polypeptide insertion (~150–200 amino acids) in their C-terminal segment immediately following the substrate-binding domain and before the C-terminal domain. A third motif, which lacks the C-terminal regulatory domain altogether, is also found in some bacteria such as *Clostridium* and including some, such as *Mycobacterium tuberculosis*, that also produce PGDH with the extended C-terminal motif. Recent studies on the parasite *Entamoeba histolytica* (7) found a variation of this third motif where the dimeric enzyme appears to have a lysine residue rather than a histidine at the active site.

The SerA1 gene from *M. tuberculosis* (H37Rv lab strain) coding for PGDH has been found to be an essential gene in mycobacterium by Himar1-based transposon mutagenesis in H37Rv strain (8). Kinetic studies (6) have revealed that *M. tuberculosis* PGDH has a Kₘ value of ~85 μM for hydroxypropyruvic acid phosphate and a kₐcat/Kₘ value of ~5.6 × 10⁶ M⁻¹ s⁻¹. Unlike *E. coli* PGDH, it displays no activity with α-ketoglutarate. In addition, *M. tuberculosis* PGDH exhibits substrate inhibition similar to what has been reported for rat liver PGDH (9). Increased ionic strength (100–400 mM) reduces the degree of substrate inhibition and shifts the maximal activity to higher substrate concentrations, also seen in rat liver PGDH. In addition, the activity of the enzyme is also dependent on the ionic strength of the buffer.

*E. coli* PGDH is strongly inhibited by l-serine (I₅₀ ≈ 2–4 μM) and classified as a V-type enzyme in which the velocity of the reaction rather than the affinity of the substrates is mainly affected (10). In *B. subtilis* (11) and *Corynebacterium glutami-
cum (12). PGDH is less sensitive to l-serine and requires extensive preincubation of the enzyme with the inhibitor. In the pea (Pisum sativum), the sensitivity to l-serine has been reported to be cold labile (13). L-Serine has been reported to be cold labile (13). L-Serine was transformed into cells. The cells were grown in M9 minimal medium with all of the 19 standard amino acids except Met. Selenomethionine (50 mg/liter) and L-Serine has been reported to have a cold lability (13) due to how this extra region is affecting the overall structure of the enzyme, which is strongly inhibited by l-serine, with an IC_{50} value for l-serine of 30 μM (6), making it the next most sensitive enzyme after E. coli to be reported. Unlike the E. coli enzyme, M. tuberculosis PGDH displays a Hill coefficient of −1.0, indicating the absence of cooperativity for serine inhibition. However, kinetic studies show that cooperativity can be induced in M. tuberculosis PGDH by the presence of chloride ions (6) and to a lesser extent by bromide and iodide.

In this study, we report the crystal structure of PGDH from M. tuberculosis in the absence of physiological ligands. This structure represents the second class of PGDH with extended motif at the C terminus. This structure provides information about how this extra region is affecting the overall structure of this class of PGDH and may eventually lead to important insights into the allosteric regulation of this protein. Remarkably, although the tetramer is composed of identical subunits, significant asymmetry is seen in the tertiary structure of the subunits.

MATERIALS AND METHODS

Cloning, Expression, and Purification of M. tuberculosis PGDH Protein—The SerA1 gene (Rv2996c) from M. tuberculosis H37Rv genome was cloned into pET30b vector (Novagen) using Ndel and HindIII sites. The Ndel site contained the ATG start codon, and the HindIII site was preceded immediately by a stop codon following the C-terminal residue of the enzyme. Production of a selenomethionylated (SeMet) protein for multiple anamolous dispersion (MAD) phasing was facilitated by mutagenesis of Ile (ATC) to position 514 to Met (ATG) using a QuickChange site-directed mutagenesis kit (Stratagene). This site was chosen because the homologous position in E. coli PGDH is a methionine residue. The mutated gene was cloned into the pET30b vector in the same way as in the wild type.

The pET30b-SerA1 clone was transformed into the E. coli overexpression strain BL21 (DE3). The culture was grown in LB medium with kanamycin (50 μg/ml) at 37 °C until the A_{600} reached 0.6–0.8 and then induced with 1 mM isopropyl-1-thio-β-D-galactopyranoside and grown at 18 °C overnight. For SeMet protein, the pET30b-SerA1 (I514M) clone was transformed into E. coli B334 (DE3) (Novagen), Met auxotrophic strain. The cells were grown in M9 minimal medium with all of the 19 standard amino acids except Met. Selenomethionine (50 mg/liter) and kanamycin (50 μg/ml) were also added to the culture. The culture was induced with 1 mM isopropyl-1-thio-β-D-galactopyranoside and then grown overnight at 18 °C. The cells were harvested by centrifugation at 3000 rpm for 30 min and resuspended in 20 mM phosphate buffer, pH 7.5, 5 mM KCl. The cells were lysed using a French Press, and the cell debris was removed by centrifugation at 15,000 rpm for 1 h. The supernatant was then loaded on to a Q-Sepharose Fast Flow column (Amersham Biosciences) that was equilibrated with 20 mM phosphate buffer, pH 7.5, 5 mM KCl, and protein was eluted using a KCl gradient. The fractions containing PGDH were pooled and concentrated by Centriprep (Millipore) and applied to a Superdex-200 gel filtration column (Amersham Biosciences) equilibrated with 100 mM phosphate buffer, pH 7.5, 1 mM dithiothreitol, 1 mM EDTA.

Crystallization—Native and SeMet PGDH (13 mg/ml) were crystallized in 1 μM NaK tartrate, 0.1 μM MES, pH 6.5 (Wizard condition) at 18 °C by vapor diffusion in hanging drops. This precipitant condition was found to produce crystals of good diffraction quality after doing a matrix screening with different kits (Hampton Crystal Screens and Wizard screens I and II from Emerald Biosystems). The crystals grew within 3–4 days in 1 μl hanging drops of 5 μl of PGDH (15 mg/ml) and 2 μl of precipitant. The same conditions were used for SeMet, but the crystals took more than a week to grow. The crystals were flash frozen in liquid nitrogen using 20% propane glycol as cryoprotectant for data collection.

Data Collection and Processing—The initial native data set for M. tuberculosis PGDH was collected at 2.8 Å at APS 14-BMC (Advanced Photon Source). Molecular replacement of high resolution data of M. tuberculosis PGDH was attempted with the E. coli PGDH monomer or its nucleotide-binding domain (the largest domain) as a model using various programs (AMORE, MOLREP, and Phaser). However, no solution was obtained. Subsequently, several different heavy metals such as mercury, platinum, and neodymium were tried but failed to produce an acceptable isomorphous or anomalous signal. SeMet MAD phasing with PGDH was originally not possible because this 529-amino acid protein did not contain any methionyl residues except the N-terminal methionine, which was cleaved during expression. Therefore, a single methionine residue was introduced at position 514 that was sufficient to obtain complete and highly redundant SeMet MAD data sets (peak, reflection) for PGDH at 3.15 Å, collected at APS 14-BMD station in Chicago. High resolution data for PGDH at 2.3 Å was also collected at APS 14-BMC in Chicago. The diffraction data sets were processed and scaled using HKL2000 (15). The space group of the crystals was determined to be P6_22. Solvent content indicated either a dimer or a trimer in the asymmetric unit. Two selenium sites were found using SHELXD (16) and further refined using AUTOshARP (17). An interpretable density modified solvent flattened map was also obtained using AUTOshARP showing two molecules in the asymmetric unit.

Structure Determination—Using this density modified map, chain tracing and automated model building was done using CAPRA in TEX-TAL (18). All of the residues of PGDH except the first two residues could be built in the electron density map. The model was then refined against the high resolution (2.3 Å) data using REFMAC (19). The refinement quality of the model was greatly improved using CNS (rigid body refinement and then simulated annealing refinement at 4000 K) (20). Several rounds of manual model building with Xtalview (21) using the Shake&SwARP (22) bias minimized electron density maps and refinement using REFMAC were performed. After the model reached a reasonable R factor, water was added to the structure using automated water picking in Xtalview as well as manual picking using unbiased electron density maps. Finally, the model was translation-libration-screw (TLS) restrained and refined using REFMAC, and the final structure has an R factor of 20.5% and an R_{free} value of 25% with good stereochemistry as analyzed by PROCHECK (23) (Table I).

All of the structure figures were prepared using PyMOL (24) except for panels A and B of Fig. 3, which were made using Raster 3D (25), and the molecular surface representations in Fig. 3 (C and D), which were made using SPORC (26).

RESULTS

The structure of M. tuberculosis PGDH has been refined to 2.3 Å resolution using SeMet MAD phasing (27). To use this method, the isoleucyl residue at position 514 was mutated to methionine. Based on sequence alignment, this position is occupied by a methionyl residue in E. coli PGDH. Although it is generally suggested that there should theoretically be one selenomethionine for 100 amino acids to yield sufficient MAD phasing (27), a complete and highly redundant data set at 3.15 Å provided an interpretable electron density map of high quality from this one Met in the 529-amino acid protein (two asymmetric unit, no NCS averaging). The structure was refined to an R factor of 20.5% and an R_{free} of 25% with good stereochemistry. The refined structure contains all the amino acid residues except the first two residues of the protein (Fig. 1A). The x-ray crystallographic structure shows that there are two molecules found in the asymmetric unit (chain A and chain B) that form a tetramer with the crystallographic symmetry related molecules (2-fold axis of symmetry). Four well defined domains can be discerned in each subunit of 55,000 Daltons (Fig. 1C). These are referred to as the substrate-binding domain (residues 3–98 and 283–319), the nucleotide-binding domain (residues 99–282), the regulatory domain (residues 454–529), and the intervening domain (residues 320–453). The substrate-binding, nucleotide-binding, and regulatory domains are homologous to those found in E. coli PGDH (Fig. 1, B and D). The intervening domain between the substrate-binding and regulatory domains is not present in E. coli PGDH. Superposition of M. tuberculosis chain B on chain A with the nucleotide-binding domain and substrate-binding domain as reference shows a rotation of the other two domains by approximately 90°.
Structure of *M. tuberculosis* PGDH

**Table I**

| Data collection | Selenium peak | Selenium inflection | Native |
|-----------------|---------------|---------------------|--------|
| Space group     | P6$_1$,22     | P6$_1$,22           | P6$_1$,22 |
| Unit cell dimensions | $a = b = 165.33$ Å $c = 217.92$ Å | $a = b = 165.4$ Å $c = 218.1$ Å | $a = b = 165.51$ Å $c = 218.14$ Å |
| Molecules/ASU$^a$ | 42           | 42                  | 42     |
| Wavelength (Å) | 0.9794  | 0.9796  | 0.9    |
| Resolution range (Å) | 141.42–3.15 | 141.42–3.15 | 48.8–2.3 |
| Highest resolution bin (Å) | 3.26–3.15 | 3.26–3.15 | 2.38–2.3 |
| Observed reflections | 777,664 | 802,306 | 1,117,427 |
| Unique reflections | 57,572 | 57,665 | 78,223 |
| Completeness (%)$^b$ | 100 (100) | 100 (100) | 99.7 (99.9) |
| Average redundancy$^b$ | 13.5 (10.2) | 13.9 (11.9) | 14.3 (9.8) |
| $I/σ(I)^b$ | 19 (3.6) | 18.7 (4.3) | 24.7 (4.8) |
| $R_{	ext{sym}}$ (%) | 0.15 (0.683) | 0.15 (0.70) | 0.095 (0.40) |
| $R_{	ext{free}}$ (%) | 8.5 | −9.3 | 2.5 |

**Refinement statistics (REFMAC)**

| Free $R$ value (%) | 24.9 |
| $R$ value (%) | 20.5 |
| No. of protein residues | 1054 |
| No. of water molecules | 315 |
| r.m.s.d. bond length (Å) | 0.012 |
| r.m.s.d. bond angles (%) | 1.47 |

**Ramachandran plot (PROCHECK)**

| Most favored region (%) | 804 (88.0) |
| Additional allowed regions (%) | 108 (11.8) |
| Generously allowed regions (%) | 2 (0.2) |
| Disallowed regions (%) | 0 |

$^a$ ASU, asymmetric unit.

$^b$ Values in parentheses for the highest resolution bin.

$^c$ $R_{	ext{sym}} = I − (I/|I|)$, where $I$ is the observed intensity, and $(I)$ is the average intensity of multiple observations of symmetry-related reflections.

$^d$ $R = |F_{	ext{calc}}| - |F_{	ext{obs}}|$, where $F_{	ext{calc}}$ and $F_{	ext{obs}}$ are the observed and calculated structure factors.

180°, indicating the presence of two different conformations among the molecules of the asymmetric unit (Fig. 1E).

**Nucleotide-binding Domain**—The nucleotide-binding domain in *M. tuberculosis* PGDH consists of residues Asn$^{29}$ to Gly$^{282}$ and contains seven parallel β strands interconnected with seven helices. This is a variation of the Rossman fold that is a characteristic of dehydrogenases. Sequence alignment of PGDH with seven helices. This is a variation of the Rossman fold that shows 33.9% sequence identity, and superimposition of the nucleotide-binding domain in *M. tuberculosis* PGDH and *E. coli* PGDH is different from either the open state exposed to solvent. NAD-dependent

**Active Site Cleft**—Similar to *E. coli* PGDH, and as seen in most of the dehydrogenases, the nucleotide-binding domain and substrate-binding domain form the active site cleft. The presence of the active site in this cleft is further supported by the presence of the nucleotide-binding site fingerprint (Gly$^{149}$ Xaa$^{150}$-Gly$^{151}$-Xaa$^{152}$-Xaa$^{153}$-Gly$^{154}$-Xaa$^{157}$-Asp$^{172}$), which is present in *M. tuberculosis* PGDH and which form a charge relay system common to many dehydrogenases and also seen in serine proteases as a His-Asp pair. Also common to dehydrogenases is a basic residue in the cleft region that serves to anchor the C-1 carboxyl group, which is Arg$^{238}$ in *M. tuberculosis* PGDH and Arg$^{240}$ in *E. coli* PGDH. Amino acid sequence alignment of PGDH with homologous proteins shows that these three residues are universally conserved. Additional homologous residues found in the cleft region are Arg$^{251}$ and Arg$^{352}$, which signifies that the residue resides on the adjacent subunit, which correspond to Arg$^{260}$ and Lys$^{241}$ in *E. coli* PGDH. Arg$^{252}$ is contributed by the adjacent nucleotide-binding domain. These residues, along with Arg$^{252}$, may play a role in the binding of the negatively charged substrate. Also apparent in *M. tuberculosis* PGDH is Trp$^{130}$, homologous to Trp$^{239}$ in *E. coli* PGDH, that is also located in the nucleotide-binding domain of the adjacent subunit and whose side chain inserts into a hydrophobic pocket near the active site of the neighboring subunit where the His-Glu pair forms the top of the pocket. This residue is thought to play an important role in subunit communication and tetramer formation in *E. coli* PGDH.

The active site clefts in this structure of *M. tuberculosis* PGDH seem to be in an open state exposed to solvent. NAD-dependent
dehydrogenases transfer a hydride ion during their catalytic process, and these hydride ions are unstable in water, so the active site must be shielded from bulk solvent during hydride transfer. This presumably unproductive state is very similar to the situation seen in the crystal structure of inhibited E. coli PGDH and seems to represent a precatalytic conformation.

**Intervening Domain**—The intervening domain (residues Val^{320} to Glu^{453}) is termed as such because it is a new domain not present in E. coli PGDH that is located between the substrate-binding domain and the regulatory domain where l-serine binds. It consists of six β strands, of which two strands are flanked by two helices and the remaining four strands form an anti-parallel β sheet. Superimposition of the intervening domains of chain B on chain A in M. tuberculosis PGDH (r.m.s.d. = 1.8 Å for Cα atoms) does not show any significant secondary structure differences. However, as noted previously, the orientation of the intervening domain in chain B is approximately 180° opposite and inverted compared with that in chain A (Fig. 1F). The average B factor values (ranging from 25 to 41 Å²) for all the domains from both the chains (A and B) are not significantly different. This inversion results in the regulatory domain of chain A occupying a position much closer to its substrate-binding domain than in chain B. These conformations will be referred to as syn- (chain A) and anti- (chain B) to distinguish them from each other for the purpose of discussion. One result of this inversion, other than the reorientation of the domains, is that there are two different interfaces between the nucleotide-binding domain and the intervening domain in the two unique subunits. Because of this, the inversion also results in the exposure of a loop region, consisting of residues 376–386 in chain B, to the solvent. This appears to lead to increased flexibility of this loop, leading to disorder in the electron density in this region.

A VAST and DALI structural alignment search using only the intervening domain found similarity with a region of domain II of 2-methylcitrate dehydratase from E. coli (Protein Data Bank Code 1SZQ; r.m.s.d. of 2.8 Å for 83 Cα atoms). This enzyme is involved in propionate catabolism and catalyzes the conversion of 2-methylcitrate to 2-methyl-cis-aconitate. Unfortunately, the role of this domain in this enzyme is not known. Apart from the numerous other hits with a Z score of 2–4.5, DALI search also found similarity with chorismate mutase in B. subtilis (Z score = 5; Protein Data Bank code 2CHS; r.m.s.d. of 3.8 Å for 83 Cα atoms) (28) that is a homotrimer with the monomer having a single domain that catalyzes the conversion of chorismate to prephenate in aromatic amino acid biosynthesis. There was also some structural similarity with uracanase hydratase from Pseudomonas putida (VAST score = 7.8; Protein Data Bank code 1UWK; r.m.s.d. of 2.3 Å for 52 Cα atoms) (29) involved in the histidine degradation pathway. Interestingly, the structural similarity is seen in the region that is part of the NAD-binding domain in this enzyme, but no such NAD-
binding fingerprint region is present in the intervening domain of the PGDH.

There are two distinct areas of electron density visible in the region where adjacent intervening domains converge. These areas of electron density are surrounded by the positively charged residues, His\[^{447}\], Lys\[^{439}\], Arg\[^{451}\], and Arg\[^{501}\]. These densities appear to represent tartrate molecules, which are included as a part of the crystallization condition. The tartrate densities appear to represent tartrate molecules, which are being trapped during crystal packing.

Whether this has any physiological significance or results from the symmetry related molecule. At present it is not known in this case, tartrate appears to reside in a pocket formed with the C-4 carboxyl group of tartrate. His\[^{447}\], which is found in the strand connecting the intervening domain and the regulatory domain, forms ionic contact with the C-1 carboxyl group of tartrate. Two molecules of tartrate bind to this region because of the symmetry of the structure and appear to form an intersubunit bonding network. Kinetic studies (6) have shown that serine inhibition of M. tuberculosis PGDH is not a cooperative process as seen for the E. coli enzyme. However, cooperativity can be induced by the presence of negatively charged ions such as chloride even at physiological concentrations. This site may represent a locus for the modulation of cooperativity of inhibition in M. tuberculosis PGDH.

There is also another region nearby in the intervening domain seen only in chain B, which also appears to bind tartrate. In this case, tartrate appears to reside in a pocket formed with the symmetry related molecule. At present it is not known whether this has any physiological significance or results from being trapped during crystal packing.

The Regulatory Domain—The C-terminal regulatory domain of M. tuberculosis PGDH consists of residues Gly\[^{454}\}–Ser\[^{529}\} and contains four anti-parallel \(\beta\) strands interconnected with two helices. It is a member of the ACT domain family, the archetypal representation of which is the regulatory domain of E. coli PGDH. ACT domain-containing proteins almost always bind a small molecule regulatory ligand associated with the ACT domain, although the locus of binding is not always analogous. ACT domains are very prevalent among enzymes that participate in amino acid metabolism, and the ACT domain has been called the "regulatory domain in amino acid metabolism" (30). The ACT domain has also been found in thiamine-binding proteins as the locus for thiamine binding (31), in the NikR protein as the locus for nickel binding (32), and in ATP phosphoribosyltransferase of M. tuberculosis (33) where it binds histidine, the end product of the pathway.

In the PGDH tetramer, two adjacent regulatory domains form an interface that creates a sheet of eight \(\beta\) strands (34). This type of arrangement is also seen in other proteins of the ACT domain family not directly involved in amino acid metabolism such as nucleoside diphosphate kinase and aspartate carbamoyltransferase, but these proteins appear to lack an allosteric site at their interfaces (31).

Superimposition of the E. coli regulatory domain on the M. tuberculosis regulatory domain (r.m.s.d. = 3.21 Å for C\(_\alpha\) atoms) shows a slight shift in one of the helices in M. tuberculosis PGDH (residues Pro\[^{497}\} and Val\[^{518}\}) because of longer loop regions in M. tuberculosis PGDH (Glu\[^{490}\}–Ala\[^{496}\} and Leu\[^{502}\}–Val\[^{506}\}) Kinetic studies on serine inhibition of M. tuberculosis PGDH show that there is a mixed type of noncompetitive inhibition with L-serine (6), so we expect to see serine binding at a site other than the active site. In the inhibited structure of E. coli PGDH, residues His\[^{344}\}, Asn\[^{346}\}, and Asn\[^{364}\} interact with L-serine, an allosteric inhibitor of PGDH, at the regulatory domain interface. The sequence alignment of M. tuberculosis PGDH with E. coli PGDH suggests that Tyr\[^{461}\}, Asp\[^{463}\}, and Asn\[^{481}\} perform the function of serine binding.

Changes in the orientation of residues Asp\[^{463}\} and Arg\[^{464}\} at this site in M. tuberculosis PGDH chains A and B of this structure (Fig. 3, A and B) have also been noticed when both the regulatory domains are superimposed (r.m.s.d. = 0.8 Å for C\(_\alpha\) atoms). With these changes in the orientation, the positions of neighboring residues (residues Val\[^{462}\} and Pro\[^{465}\}–Gly\[^{469}\}) in chain B have also been affected. In these orientations, Arg\[^{464}\} forms a hydrogen bond with Glu\[^{489}\} in chain A and with Asp\[^{519}\} in chain B. There is also change in orientation of the side chain of His\[^{460}\} in chain B, which now forms a hydrogen bond with Asp\[^{490}\}. This in turn has changed the orientation of side chain of Tyr\[^{521}\} in chain A. Asp\[^{490}\} interacts with Arg\[^{446}\}, whose side chain is oriented away from the Asp\[^{490}\} in chain B.

At the regulatory domain interface, the proposed serine-binding site does not seem to be accessible to solvent. For serine to access its binding site, either the adjacent regulatory domains must separate from each other to some extent without completely dissociating, or a channel to the surface large.
enough for serine to pass must form. This orientation change of Asp$^{463}$ and Arg$^{464}$ at the serine-binding site in one subunit might indicate a path for serine entry. Indeed, a molecular surface representation of the regulatory domain interface (Fig. 3, C and D) reveals an apparent channel in subunit A but not in subunit B. However, an answer to the question of what is triggering this orientation change in one subunit of an apoenzyme, but not the other, is not clear.

**Intrasubunit Domain Contacts**—The inversion of the two unique subunits at the connection between the substrate-binding domain and the intervening domain results in two different and unique sets of intrasubunit interactions between the nucleotide-binding domains and the intervening domains. These are listed in Table II and consist of the unique interactions between the nucleotide-binding domain and the intervening domain of subunit B (and D) and the nucleotide-binding domain and intervening domain of subunit A (and C). A diagram comparing the subunit and domain arrangement of *E. coli* and *M. tuberculosis* PGDH is shown in Fig. 4.

**Intersubunit Domain Contacts**—In the *E. coli* PGDH structure, there are only two types of intersubunit contacts, those between adjacent nucleotide-binding domains and those between adjacent regulatory domains. This structure shows that intersubunit domain contacts are more extensive in *M. tuberculosis* PGDH. These are listed in Table III and diagrammed in Fig. 4. They consist of interaction between the nucleotide-binding domain of subunit B (and D) and the intervening domain of subunit A (and C). A diagram comparing the intersubunit domain arrangement of the two enzymes is shown in Fig. 4.

**DISCUSSION**

The tetrameric *M. tuberculosis* PGDH is composed of identical subunits and presumably contains four active sites and four effector binding sites. The effector binding sites are tentatively located at the interface between regulatory domains by homology to *E. coli* PGDH. The active sites appear to be located in the clefts formed by the nucleotide- and substrate-binding domains, again homologous to *E. coli* PGDH. This is substantiated by the presence of the NAD-binding fingerprint in this region. Interestingly, *E. coli* PGDH copurifies with two tightly bound NADH molecules. This does not seem to be the case in this enzyme because no density for NADH is seen. In addition, there also appears to be four additional binding sites for a small negatively charged molecule where the intervening and regulatory domains from adjacent subunits come together. In this structure, the sites are occupied by tartrate molecules, but these could be the sites of interaction with chloride ion that has been shown kinetically to modulate the cooperativity of inhibition (6). Chloride ion may not be the physiological ligand for these sites because it is monovalent and the site contains basic side chains optimally arranged to bind a multivalent anion. On the other hand, chloride may show its effect by disrupting the ionic bridging interaction at this site seen with tartrate or whatever the actual physiological ligand may be. However, the chloride effect is not strictly ionic because raising the ionic strength of the buffer with other salts is not effective.

There are two major points of interaction between the adjacent subunits in the tetramer. One, which is very extensive, is
tory domain acts as a hinge region that is important for serine binding and inhibition. In *M. tuberculosis* PGDH the connection between the substrate-binding domain to the regulatory domain is interrupted by the presence of the intervening domain. However, like *E. coli* PGDH, the connecting strand coming from the substrate-binding domain to the next domain contains a sequence of multiple glycyl residues, in this case three (residues Gly316–Gly318). Therefore, a similar type of hinge mechanism may be operating in *M. tuberculosis* PGDH. It is not possible to tell what the significance of this may be without further experimental evidence, but one scenario may be that the intervening and regulatory domains are acting as a single unit in *M. tuberculosis* PGDH, like an extended regulatory domain, with the functional poly-glycyl hinge being found in homologous locations in each enzyme. If this is the case, common features between the association of the regulatory domain with the substrate-binding domain in *E. coli* PGDH and the intervening and substrate-binding domain in *M. tuberculosis* PGDH may lead to new insights in the pathway of allosteric inhibition of the active site.

The overall arrangement of subunits in this tetramer is strikingly different from that seen in *E. coli* PGDH. The presence of the extra 135 amino acid polypeptide (the intervening domain) has led to the movement of the whole regulatory domain toward the nucleotide-binding domain forming a curved structure in each subunit as compared with the elongated form seen in *E. coli* PGDH. There is also the presence of asymmetry in the tetramer because of the shifting of this regulatory domain interface from the extreme ends as in an elongated *E. coli* PGDH tetramer to the sides of a more globular *M. tuberculosis* PGDH tetramer, which is very unique.

In addition, the extreme asymmetry observed in the orientation of the intervening and regulatory domain in the two unique subunits is quite remarkable. Essentially, one half of the molecule rotates ~180° relative to the other half to produce a completely different domain geometry in one subunit relative to the other. The locus of rotation is in the loop connecting the substrate-binding domain to the intervening domain. An additional result of the inversion of these domains is that two different areas of the intervening domain face the nucleotide-binding domain forming two different subunit specific domain interfaces. In the syn-conformation the area of the intervening domain that faces the nucleotide-binding domain includes residues Ser375–Thr397. In the anti-configuration, these residues are exposed to solvent, and residues Gly355–Glu361 face the nucleotide-binding domain.

Structural asymmetry has been noted in other protein structures composed of homodimers or homotetramers. However, this asymmetry usually involves smaller structural elements such as loops found near the active site. The asymmetry seen here, involving large changes in the orientation of whole domains, seems unprecedented. This asymmetry also raises important and interesting questions regarding the mechanism of effector inhibition. L-Serine, the inhibitor, presumably binds to the regulatory domain of each subunit in a manner similar to that in *E. coli* PGDH. In *M. tuberculosis* PGDH the location of these subunits relative to the active site is completely different in two of the subunits relative to the other two. So, are there different mechanisms for transduction of signal to the active site functioning in each structure, or can the same mechanism of transduction be induced similarly by the two different structures? On the other hand, if *M. tuberculosis* PGDH exhibits half-of-the-sites activity like *E. coli* PGDH, are there only two subunits that are functional, whereas the other two with the different domain arrangement are not? This holds intriguing possibilities for gaining insight into the mechanism of intermo-

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**TABLE III**

| Intersubunit domain contacts | Distances (Å) |
|-------------------------------|---------------|
| **Nucleotide binding domain of B (D)** | **Intervening domain of C (A)** | **Distance** |
| Glu153 NE2 | Glu305 OE1 | 2.66 |
| Glu182 NE2 | Arg310 O | 2.55 |
| Glu182 O | Glu305 N | 3.12 |
| Glu182 OE1 | Ser346 N | 2.99 |
| **Nucleotide binding domain of A (C)** | **Intervening domain of C (A)** | **Distance** |
| Gln182 NE2 | Ser345 OG | 3.05 |
| **Intervening domain of A (B)** | **Intervening domain of D (C)** | **Distance** |
| Asn408 OD1 | His399 ND1 | 3.22 |
| His409 ND1 | Asn398 OD1 | 2.72 |
| Arg410 N | Asn397 O | 3.33 |
| Arg410 NH2 | Ser346 O | 2.8 |

 FIG. 4. Comparison of the subunit and domain interaction in *E. coli* and *M. tuberculosis* PGDH. Subunits are depicted as geometric shapes. Parallelograms are used for the similar conformations in *E. coli* PGDH and rectangles (syn) and trapezoids (anti) for the two distinct conformations in *M. tuberculosis* PGDH. The subunits are labeled A–D as in the text. N, nucleotide-binding domain; S, substrate-binding domain; I, intervening domain; R, regulatory domain. Solid lines depict intersubunit interactions, and dotted lines depict intrasubunit interactions between domains that are not contiguous in polypeptide sequence.
lecular signal transduction. These questions can only be answered with additional information. In this regard, mutagenesis experiments designed to lend insight into the mechanism of this very interesting protein are presently underway.

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