Archives of Biochemistry and Biophysics 570 (2015) 32–39

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

# Comparison of Type 1 D-3-phosphoglycerate dehydrogenases reveals unique regulation in pathogenic *Mycobacteria*

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ARTICLE INFO

*Article history:*
Received 15 December 2014
and in revised form 30 January 2015
Available online 16 February 2015

*Keywords:*
Phosphoglycerate
Dehydrogenase
L-Serine
Mycobacteria
Feedback inhibition

ABSTRACT

D-3-phosphoglycerate dehydrogenases (PGDH) from all organisms catalyze the conversion of D-3-phosphoglycerate to phosphohydroxypyruvate as the first step in the biosynthesis of L-serine. This investigation compares the properties of Type 1 PGDHs from seven different species and demonstrates that conserved residues in the ACT and ASB domains of some allow L-serine to act as a feedback inhibitor at low micromolar concentrations. In addition, the serine sensitivity is dependent on the presence of phosphate ions. These residues are most highly conserved among PGDHs from the actinomycetales family, but only certain pathogenic mycobacteria appear to have the full complement of residues required for high sensitivity to serine. These basic residues are also responsible for the presence of dual pH optima in the acidic region that is also phosphate dependent. Analytical ultracentrifugation analysis demonstrates that the dual pH optima do not require changes in oligomeric state. This study also demonstrates that substrate inhibition is a common feature of Type 1 PGDHs and that it is suppressed by phosphate, indicating that phosphate likely interacts at both the catalytic and regulatory sites. The unique features resulting from the complement of basic residues conserved in pathogenic mycobacteria may impart important metabolic advantages to these organisms.



## Introduction

D-3-phosphoglycerate dehydrogenase (PGDH[1], EC 1.1.1.95) is an essential metabolic enzyme in bacteria, plants, and animals [1–4]. It is the first enzyme in the L-serine biosynthetic pathway and catalyzes the conversion of the glycolytic intermediate, D-3-phosphoglycerate (PGA), to phosphohydroxypyruvate (PHP). PHP is subsequently converted to phosphoserine by phosphoserine aminotransferase (PSAT) and then phosphoserine is irreversibly converted L-serine by phosphoserine phosphatase (PSP). Although PGDH catalyzes the same reaction in all organisms, not all PGDH enzymes are alike. Three types of PGDH have been observed that differ in their size and domain composition [5,6]. The largest are termed Type 1 PGDH and contain an ACT domain [7] and an ASB domain [6] in addition to a catalytic domain. The Type 2 PGDHs contain only an ACT domain in addition to the catalytic domain while Type 3 PGDH consists only of the catalytic domain. The catalytic domains of all three types are structurally homologous although they differ somewhat in length at their amino termini.

Although it is widely reported that PGDH is feedback inhibited by L-serine, it is not the case for all PGDHs and when it does occur the reports vary to a large degree on the sensitivity of the inhibition. In particular, it has recently been demonstrated that the Type 1 PGDH from *Mycobacterium tuberculosis* is optimally inhibited by L-serine only in the presence of phosphate ions [8]. On the other hand, PGDH from *Bacillus subtilis* [9] and *Corynebacterium glutamicum* [10] are Type 1 enzymes that have been reported to be relatively insensitive to L-serine with reported $IC_{50}$ values of approximately 5 mM and 10 mM, respectively. Type 1 PGDHs from vertebrate sources, such as rat and human, show no sensitivity to L-serine [11,12]. Since many reports use buffers that are devoid of phosphate ion, it is not clear how phosphate may affect their activity.

The pH rate profile of *M. tuberculosis* PGDH also displays a dual pH optimum where catalytic activity is suppressed between pH 5.5–6.5 [13]. Mutation of the cationic side chains at the allosteric substrate binding site in the ASB domain either eliminated the dual

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[1] *Abbreviations used:* PGDH, D-3-phosphoglycerate dehydrogenase; PHP, phosphohydroxypyruvate (also called hydroxypyruvic acid phosphate, HPAP); ACT, an acronym for Aspartate kinase, Chorismate mutase, TyrA; ASB, an acronym for allosteric substrate binding.

http://dx.doi.org/10.1016/j.abb.2015.02.008
0003-9861/

pH optimum or widened the suppressed area between the optima, depending on which residue was mutated. It was also found that mutation of all the cationic side chains on one of the adjacent ASB domains not only eliminated the dual pH optima, but also significantly reduced the level of substrate inhibition and completely eliminated the ability of L-serine to inhibit catalytic activity [13,14].

The X-ray structure of *M. tuberculosis* PGDH crystallized in 1 M MES buffer, pH 6.5, containing 1 M tartrate revealed a molecule of tartrate bound at the interface of ASB domains between adjacent subunits [15] (Fig. 1). The carboxyl groups of the tartrate formed ionic interactions with cationic side chains from both subunits. Since tartrate is a structural analog of the substrate/product of PGDH, phosphoglycerate/phosphohydroxypyruvate, it was suggested that this was a second, allosteric site for substrate binding.

The unusual properties of *M. tuberculosis* PGDH and the reports of widely varying sensitivity to L-serine of Type 1 PGDHs from other organisms suggested that *M. tuberculosis* PGDH may be unique and that these characteristics may impart the organism with some physiologic advantage within the context of its unique life-style. It was therefore of considerable interest to investigate whether the characteristics of *M. tuberculosis* PGDH described above were shared by other Type 1 enzymes. In this investigation, we have cloned and characterized several other Type 1 PGDHs from both pathogenic and non-pathogenic mycobacteria as well as PGDHs from more distantly related organisms. The results show that the dual pH optima in the acidic region of the pH rate profile and the dependence of L-serine sensitivity on the presence of phosphate ions are unique to the pathogenic mycobacteria used in this study. In addition, the occurrence of the dual pH optima is not due to differences in the association state of the protein.

## Experimental procedures

All proteins were expressed with a hexa-histidine tag at the amino terminus by placing them into the 5′ BamH1 site in pSV281. PGDH from *M. tuberculosis*, *Mycobacterium marinum*, *Mycobacterium smegmatis*, *Streptomyces coelicolor*, *B. subtilis*, *C. glutamicum*, were cloned by PCR from chromosomal DNA using primers matching the 5′ and 3′ nucleotide sequences of the genes with appropriate restriction sites for placement into the pSV281 expression vector. cDNA for human liver PGDH was kindly provided by Dr. Burton Wice. Proteins were expressed in BL21 DE3 cells and purified using Talon® cobalt based immobilized metal affinity columns as previously described [8]. Enzyme activity was measured in the specified buffer with phosphohydroxypyruvate (PHP) and NADH as substrates by following the change of absorbance at 340 nm. Both substrates and all buffers were from Sigma Chemical Co. PHP is purchased as the dimethylketal tricyclohexylammonium salt and converted to PHP according to the instructions from the manufacturer. It is stored frozen and thawed just prior to use.

Plots of activity versus substrate concentration were fit to the general equation for substrate inhibition [16],

$$v = \frac{V_m + V_i([S]^x/K_i^x)}{1 + (K_m^n/[S]^n) + ([S]^x/K_i^x)} \quad (1)$$

where $v$ is the velocity at substrate concentration [$S$], $V_m$ is the maximum velocity, $K_m$ is the apparent Michaelis constant, $V_i$ is the maximum inhibited velocity and $K_i$ is the inhibition constant. The exponents $n$ and $x$ allow for cooperativity of substrate binding in the catalytically productive and inhibitory mode, respectively. To obtain convergence for Eq. (1), the values of $x$ and $n$ must be fixed. The integer values that produce the best fit were determined empirically.

When complete inhibition is observed and there is no cooperativity, $V_i$, $n$, and $x$ equal zero, and the equation reduces to the standard equation for complete substrate inhibition,

$$v = \frac{V_m}{1 + (K_m/[S]) + ([S]/K_i)} \quad (2)$$

Serine inhibition is plotted as fractional inhibition, $I = (v_0 - v_i)/v_0$, versus serine concentration and fit to a Hill equation,

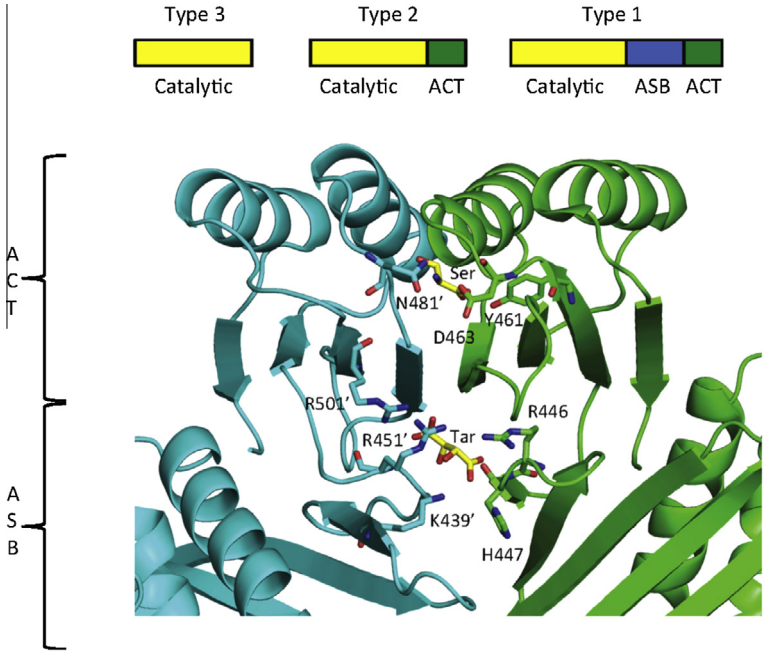


**Fig. 1.** The ASB/ACT domain interface of *M. tuberculosis* PGDH. The ASB/ACT domain interface (pdb 1YGY) shows the bound serine (Ser) and tartrate (Tar) molecules in yellow as well as the residues that bind the tartrate molecule, K439′, R446, H447, R451′, and R501′, and the residues that interact with serine, Y461, D463 and N481′. The prime designates residues on the adjacent subunit. Only one site per dimer is shown for clarity. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)