

## Automatic Structure Solution of *Bacillus Stearotherophilus* Phosphoglycerate Mutase

G. Krishnasamy, M.J. Jedrzejas (U. Alabama at Birmingham), P. Setlow, and M. Chander (U. of Connecticut)

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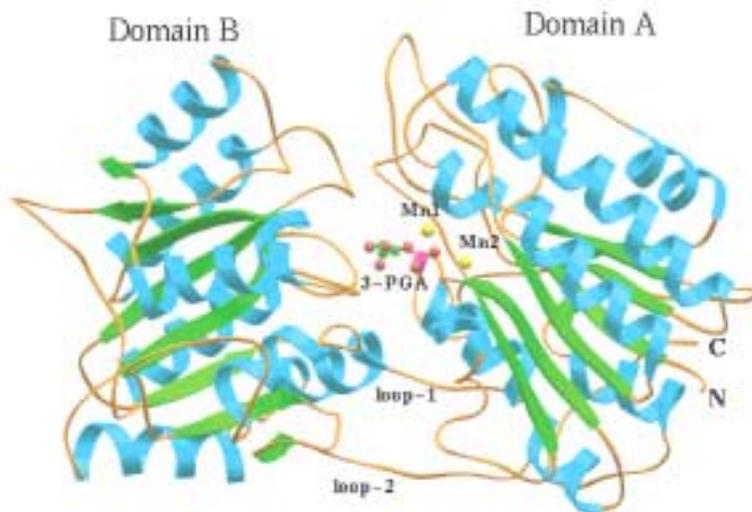
Beamline(s): X25

Phosphoglycerate mutase (PGM), an important enzyme in the glycolytic pathway, catalyzes the transfer of a phosphate group between the 2 and 3 positions of glyceric acid. The PGMs from endospore forming gram-positive bacteria such as the various *Bacillus* and *Clostridium* species have significant novel features in that the catalytic activity of these enzymes absolutely and specifically requires  $Mn^{2+}$  and is also extremely pH sensitive (1). These two properties appear both related and important in regulating the activity of these enzymes during sporulation and spore germination in *Bacillus* species (1).

Diffraction quality crystals of the *Bacillus stearotherophilus* 2,3-diphosphoglycerate independent and monomeric PGM (57 kDa) were obtained at neutral pH in the presence of 3-phosphoglyceric acid with ammonium sulfate as the precipitating agent; these crystals diffract X-rays to beyond 1.9 Å resolution and belong to the orthorhombic space group  $C22_1$  with unit cell dimensions,  $a = 58.42$ ,  $b = 206.08$ ,  $c = 124.87$ . The selenomethionyl version of the *B. stearotherophilus* protein has also been crystallized (2).

Employing the selenomethionyl crystals, the multiwavelength anomalous dispersion (MAD) X-ray data have been collected at three wavelengths at X25 beamline at NSLS. Localization of the selenium atoms and the initial phases has been obtained using the automatic procedures of Solve. After applying density modifications using CCP4 DM methodology the resultant electron density map has been traced automatically by the ARP/wARP program.

The structure comprises residues 3 to 510 (507 residues) out of 511 amino acids of a mature protein and 180 ordered water molecules (3). Single residues at the N-and C-terminus are poorly ordered and due to this are not included in the current model. The electron density is of high quality allowing for the good definition of the model. The central buried core of the structure is especially well defined as judged by good electron density and low temperature factors in the final model. The iPGM polypeptide chain adopts a compact, globular shape with two domains, A and B (Fig.1). The two domains are similar in size with approximate dimensions 24 x 35 x 31. In domain A there is only one out of eight anti-parallel  $\beta$ -strand whereas in domain B there are two parallel strands that are anti parallel to the remaining parallel six  $\beta$ -strands. These  $\beta$ -sheet structures are located in the core of each domain and are surrounded by helices of various lengths on the outside. The overall topology of the protein can be described as an alpha/beta type structure. Domains A and B are bridged by two loops, loop 1 and 2, of 7 and 11 residues long, respectively. A well defined cleft is present in-between these domains. This cleft is highly solvent accessible and more water molecules are found here as is the substrate and two manganese ions binding sites (Fig. 1).



**Figure 1.** Ribbon drawing of the *B. stearotherophilus* iPGM. The  $Mn^{2+}$  ions and the 3-PGA substrate molecule are shown in a ball and stick fashion.

**References:** M. Chander, *et al.* The enzymatic activity of phosphoglycerate mutase from gram-positive endospore-forming bacteria requires  $Mn^{2+}$  and is pH sensitive. *Can. J. Microbiol.* 44, 759-767, (1999). 2. M. Chander, *et al.* Structural studies on a 2, 3- diphosphoglycerate independent phosphoglycerate mutase from *Bacillus stearotherophilus*. *J. Struct. Biol.* 126, 156-165, [999]. 3. Jedrzejas, *et al.* Structure and mechanism of action of a novel phosphoglycerate mutase from *Bacillus stearotherophilus* *EMBO J.* 19, 1419-1431, (2000).