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Review

Structure, function, and evolution of phosphoglycerate mutases: comparison with fructose-2,6-bisphosphatase, acid phosphatase, and alkaline phosphatase

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Contents

Abbreviations: AcPase, acid phosphatase; AlPase, alkaline phosphatase; ATP, adenosine 5′-triphosphate; 13PG, 1,3-diphosphoglyceric acid; 23PG, 2,3-diphosphoglyceric acid; Fru26P2, fructose-2,6-biphosphate; Fru26P2ase, fructose-2,6-biphosphatase; 6PFru-2-K/Fru26P2ase, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase; 2PGA, 2-phosphoglyceric acid; 3PGA, 3-phosphoglyceric acid; PGM, phosphoglycerate mutase; bPGM, bisphosphoglycerate mutase; dPGM, 23PGA dependent PGM; iPGM, 23PGA independent PGM.

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1. Introduction

Phosphoglycerate mutase (PGM) enzymes catalyze the isomerization of phosphoglycerate substrates, a process essential for the metabolism of glucose and/or 2,3-phosphoglycerate (23PGA) in nearly all organisms (Fothergill-Gilmore and Watson, 1989). At least two known distinct classes of PGM enzymes were identified and one of them catalyzes the interconversion of 3-phosphoglycerate (3PGA) and 2-phosphoglycerate (2PGA). This enzyme is known as monophosphoglycerate mutase (mPGM) (EC 5.4.2.1) (Meyerhof and Kiessling, 1935) in contrast to a bisphosphoglycerate mutase (bPGM) (EC 5.4.2.4/EC 3.1.3.13) which catalyzes primarily the interconversion of 1,3-phosphoglycerate (13PGA) and 23PGA (Forthergill-Gilmore and Watson, 1989). The second enzyme is a member of another class of PGMs and this group of enzymes also has the ability to carry out the reaction catalyzed by mPGMs as well as the break down of 23PGA to 3PGA (Rapport and Luebring, 1950). This form of the PGM enzymes was isolated from erythrocytes of many vertebrates (Forthergill-Gilmore and Watson, 1989). The mPGM enzymes are divided into two main groups, the ones dependent on 23PGA as a cofactor (dPGM) and the ones independent of 23PGA (iPGM).

The dPGM enzymes are commonly identified among all vertebrates, fungi such as *Saccharomyces cerevisiae*, and in some bacterial organisms such as *Haemophilus influenzae* whereas the iPGM enzymes are specific for all plants, selected invertebrates, algae, and some bacteria such as *E. coli* or *B. subtilis* (Fothergill-Gilmore and Watson, 1989; Fraser et al., 1999; Singh and Setlow, 1979). Some bacterial organisms with larger genomes like *E. coli* or *B. subtilis*, however, have both types of mPGM, dPGM and iPGM, but only one form is predominantly active (Fraser et al., 1999; Singh and Setlow, 1979). There is no sequence similarity between the iPGMs, and dPGMs or bPGM enzymes (Singh and Setlow, 1979; Watabe and Freese, 1979; Fothergill-Gilmore and Watson, 1989; Fothergill-Gilmore and Michels, 1993; Leyva-Vazquez and Setlow, 1994; Grana et al., 1992, 1995). The dPGM enzymes have been identified in monomeric, dimeric or tetrameric forms having a molecular weight of approximately 27 kDa per monomer (Fig. 1). Sequence analysis of all known dPGMs showed that these enzymes are similar across the species. They also exhibit significant sequence and functional similarity to the bPGM enzymes which is consistent with the data showing that dPGMs can perform the same reactions as the bPGMs but at very different rates (Rose, 1980). The overall percent homology among dPGMs/bPGMs analyzed in Fig. 1 is 36% with the identity between individual pairs varying from 49% to as high as 98%.

In contrast to dPGMs, the iPGM enzymes are monomers and are significantly larger than dPGMs or bPGMs, with a molecular weight of ~50 kDa per monomer (Chander et al., 1999). The sequence similarity among iPGMs, even from different kingdoms, is very high suggesting their structural and functional similarity as well as a common evolutionary ancestor (Grana et al., 1995). They also have been identified to have some sequence similarities with alkaline phosphatases but only over a limited part of the active site residues responsible for metal binding (Galperin et al., 1998; Chander et al., 1999; Jedrzejas et al., 2000a; Jedrzejas and Setlow, 2000). The studies of the catalytic properties of the iPGM enzymes were less advanced relative to the dPGMs primarily due to a lack of their structural information (Blattler and Knowles, 1980; Breathnach and Knowles, 1977; Britton et al., 1971; Gatehouse and Knowles, 1977; Leadlay et al., 1977). Recently, however, the first structure of the iPGM enzyme from *Bacillus stearothermophilus* has been obtained (Jedrzejas et al., 2000a). Using this structural information, together with structure-guided site-directed mutagenesis, the mechanism of catalysis of this enzyme has also been elucidated and was shown to involve a phosphoserine intermediate with a two-step catalytic process involving phosphatase and phosphotransferase activity (Jedrzejas et al., 2000a). In addition, this enzyme was clearly linked in its properties to the *E. coli* alkaline phosphatase (AlPase) and other members of the alkaline phosphatase family of enzymes although only in the active site area responsible for the phosphatase activity or metal-binding residues, and not the phosphotransferase activity (Galperin et al., 1998; Jedrzejas and Setlow, 2000). Although both iPGM and AlPase are binuclear metalloenzymes (Dismukes, 1996), the *E. coli* and *B. stearothermophilus* iPGMs are manganese dependent (Fraser et al., 1999; Chander et al., 1998; Singh and Setlow, 1978) whereas the *E. coli* AlPase is zinc dependent (Jedrzejas et al., 2000a; Jedrzejas and Setlow, 2000; Coleman, 1992; Kim and Wyckoff, 1991; Ried and Wilson, 1976; Applebury et al., 1970; Petitclerc et al., 1970). It is also very likely that all iPGMs are Mn^{2+}- dependent, though more evidence is needed to support this conclusion. For a review of the iPGM structure and catalysis as well as its comparison to the alkaline phosphatase family of enzymes see a recent review by Jedrzejas and Setlow (2000).

Studies of the catalysis of dPGM enzymes (Jones et al., 1978) advanced significantly sooner than those of iPGMs due to the availability of the three-dimensional structure of this enzyme from *Saccharomyces cerevisiae* (Campbell et al., 1974). This structure was recently obtained at an increased resolution of X-ray diffraction, 2.3 Å, by Ridgen et al. (1998) followed by the structure of the protein complexed with two sulfate ions (Ridgen et al., 1999) and, finally, with the 3PGA substrate-enzyme complex at 1.7 Å resolution of diffraction (Crowhurst et al., 1999).

2. Cofactor dependent *Saccharomyces cerevisiae* phosphoglycerate mutase

2.1. Structural aspects of Saccharomyces cerevisiae phosphoglycerate mutase

The yeast enzyme has a tetrameric form similar to other dPGM enzymes which are also tetramers or sometimes dimers or monomers. The structure of this tetrameric dPGM enzyme has an arrangement of a dimer of dimers with 246 protein residues, 27 kDa, per monomer. Typical of glycolytic enzymes, every monomer has an α/β fold with a three layer sandwich consisting of a largely parallel β-sheet core flanked on both sides by α-helices (Fig. 2(a))

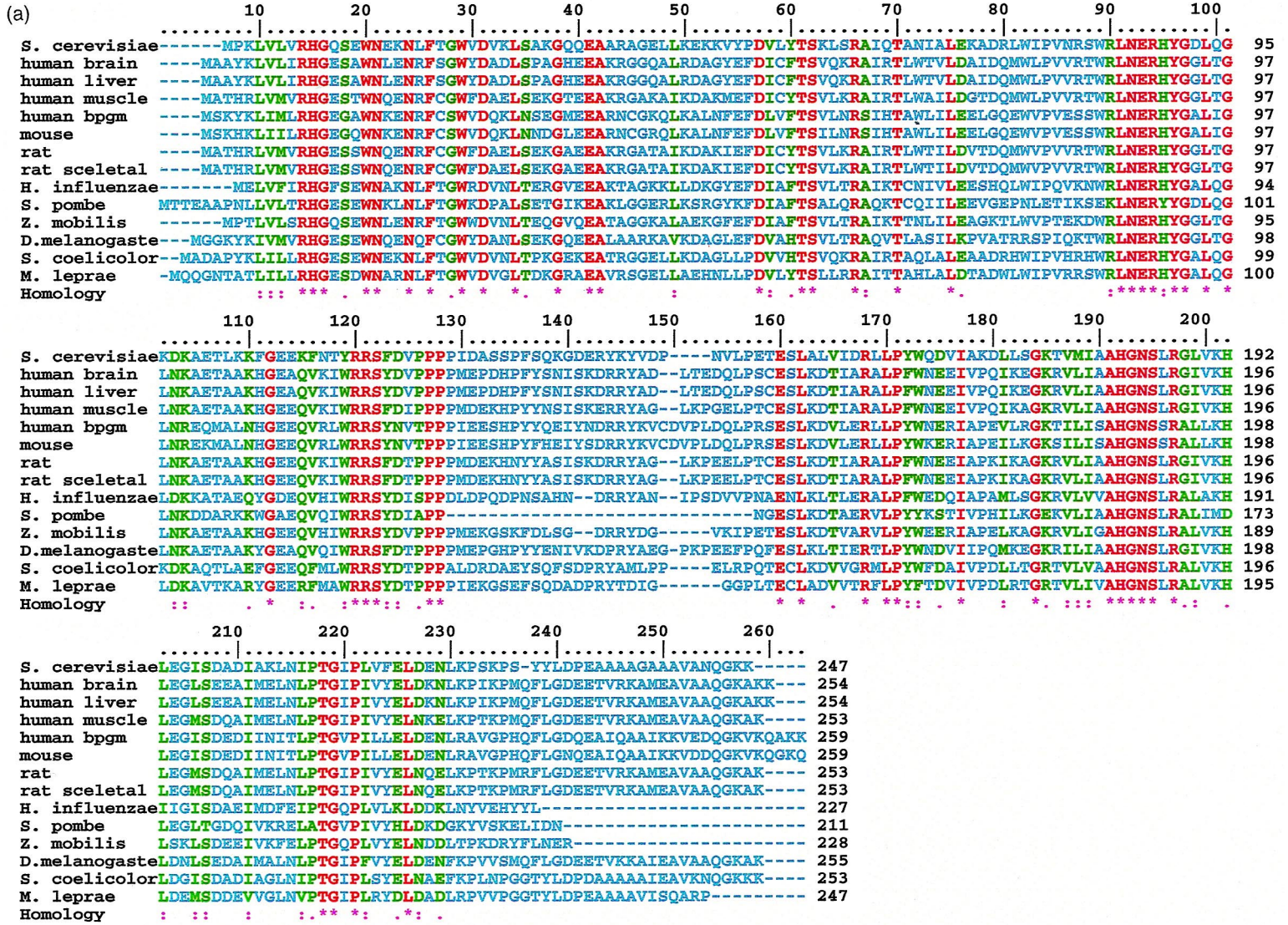

Fig. 1. (a) Sequence alignment of phosphoglycerate mutase enzymes. The source of sequence data is as follows: 1 — *S. cerevisiae*, PDB: 1QHF and PIR1:PMBYY; 2 — human brain, PIR1:PMHUYB; 3 — human liver, PIR1:PMHUBM; 4 — human muscle, PIR1:PMHUYM; 5 — human bPGM, GB_PR1:HUMPGAMMG; 6 — mouse, PIR1:PMMSBM; 7 — rat, PIR1:PMRTYM; 8 — rat skeletal, PIR2:JC1132; 9 — *Haemophilus influenzae* (strain Rd KW20), PIR2:A64091; 10 — fission yeast (*Schizosaccharomyces pombe*), PIR2:S43214; 11 — *Zymomonas mobilis*, PIR2:C40649; 12 — fruit fly (*Drosophila melanogaster*), PIR2:S50326; 13 — *Streptomyces coelicolor*, GB_BA1:STMPGM; 14 — *Mycobacterium leprae*, PIR2:S72904. (b) Sequence alignment of the *S. cerevisiae* dPGM enzyme (PDB: 1QHF) with rat liver fructose-2,6-biphosphatase (PDB: 1FBT) and rat prostatic acid phosphatase (PDB: 1RPA). The alignment was accomplished using Clustal W version 8 (Thompson et al., 1994) and displayed with Multiple Protein Sequence Alignment (MPSA) software (Blanchet et al., 1999).

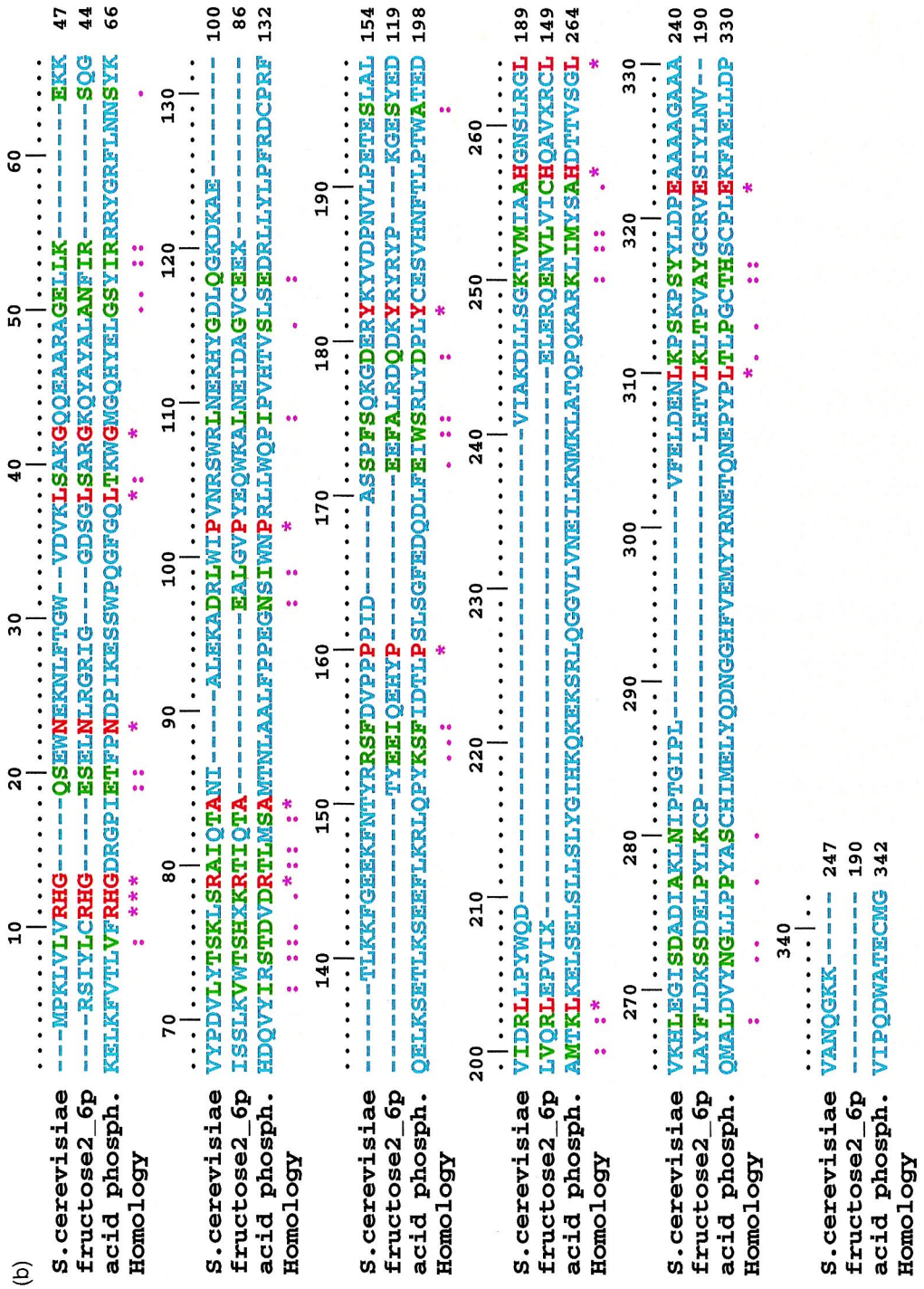

Fig. 1 (*continued*)

(Campbell et al., 1974; Ridgen et al., 1998). However, every monomer is built from two domains with the larger domain having a nucleotide-binding fold that was thought not to have any physiological relevance (Campbell et al., 1974; Winn et al., 1981). However, charged ligands other than nucleotides, such as the phosphoglycerates (23PGA, 3PGA, 2PGA), can likely bind in this location (Bazan and Fletterrick, 1990). Not surprisingly, the active site of this dPGM was located in this area by Winn et al. (1981). There is one active site per monomer located in a crevice at the C-terminal end of the β-sheet and this active site utilizes residues only from this monomer. The active site has, among other amino acids, two histidine residues, His8 and His181, with the His8 involved in forming a phosphohistidine–enzyme intermediate as a part of the catalytic cycle of this dPGM enzyme (Fig 2(b)) (Winn et al., 1981; Fothergill-Gilmore and Watson, 1989; Rose 1971, 1980; Nairn et al., 1995). The precise determination of all residues involved in the activity is still sketchy at this time due to the flexibility of at least part of the residues playing the catalytic role. Specifically, the C-terminal part containing 14 residues of the enzyme has a large degree of flexibility and its presence was not observed in any of the structures reported to date, except for the recent complex structure with 3PGA substrate where a part of this tail was located (Crowhurst et al., 1999). Based on the later studies, this part of the enzyme is seen to be crucial for activity as it partially covers the active site area, possible only during the phosphate transfer between the 2 and 3 positions of the glycerate during the catalytic process. This C-terminal tail interacts with the substrate which stabilizes the so called 'cap or lid' formation (Crowhurst et al., 1999). Therefore, the access to the active site is regulated by the conformation of the C-terminal tail of the enzyme. Site-directed mutagenesis of the very similar human bisphosphoglycerate mutase performed on the amino acid in this region of the enzyme showed the reduction of the enzyme's catalytic activity (Garel et al., 1989). Also, the proteolytic removal of these residues caused significant decrease of activity (Sasaki et al., 1966; Price et al., 1985). Based on the complex structures of the enzyme with 3PGA, residues other than His8 and His181 that interact with the 3PGA substrate and, therefore, get implicated in the activity, are as follows: Arg7, Ser11, Asn14, Arg59 (Fig 2(b), Table 1) (Crowhurst et al., 1999). However, other structural data available show two sulfate ions in the yeast dPDM's active site that seem to mimic the positions of the two phosphate ions of the 23PGA cofactor/substrate and/or the reaction intermediate of this enzyme (Ridgen et al., 1999). These two sulfate ions interact with additional residues of the enzyme: Thr20, Arg87, Tyr89, Arg113, Arg114, and Asn183 (Fig 2(b), Table 1). Based on the limited structural data of this enzyme complexes, the active site of the yeast dPGM, defined as all residues interacting with the substrate/product or the cofactor, includes all 12 above mentioned residues (Table 1). The His181 residue does not interact with 3PGA or any of the sulfate ions. Based on the sequence comparison of sequence data available for the dPGMs as well as for the bPGM enzymes, all these residues except Thr20 are strictly conserved which reinforces the importance of these residues for catalysis. Other residues in the position of the nonconserved Thr20 are, however, a very similar nature and include either Ser or Cys (Fig. 1(a)). As one might expect, the residues important for catalysis should be conserved in evolution. This definitely is the case for the iPGM enzymes known to date, where all 15 identified active site residues are strictly conserved even for organisms across different kingdoms (Jedrzejas et al., 2000a).

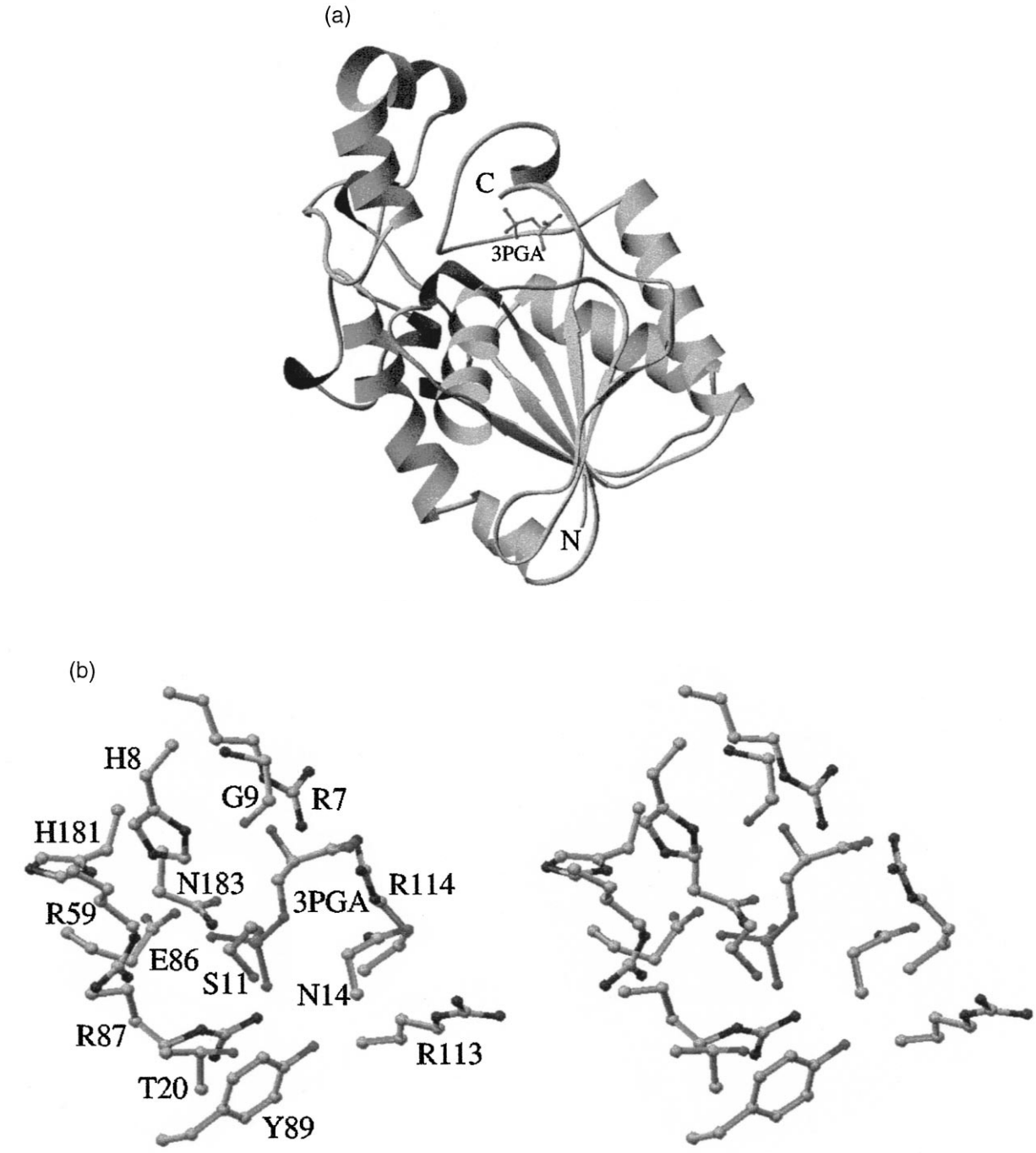

Fig. 2. (a) General structure of *S. cerevisiae* dPGM. The structure of the yeast dPGM is based on its X-ray structure (Crowhurst et al., 1999) (PDB: 1QHF). (b) Active site residues of *S. cerevisiae* phosphoglycerate mutase. The interactions of the substrate/product with the protein are shown as determined by Crowhurst et al. (1999) (PDB: 1QHF).

2.2. Mechanism of catalysis of the S. cerevisiae dPGM

The catalytic mechanism of this enzyme was thought to involve two histidine residues, His8 and His181, and proceed through a phosphohistidine–enzyme intermediate utilizing His8 (Rose, 1971, 1980; Han and Rose, 1979; Nairn et al., 1995). The role of His8 and the formation of the intermediate utilizing this residue have been well established. However, the role of the conserved His181 residue in all known dPGMs and bPGMs is still not clear, but it was originally thought to act as a proton donor/acceptor (acid/base catalyst) during the suspected catalysis through the phospho–His8 intermediate and/or the suggested hydrolysis of the phosphate group (Rose, 1980). The important role of this residue has been confirmed by site-directed mutagenesis (White and Fothergill-Gilmore, 1992; White et al., 1993a) which for the yeast enzyme leads to activity below 5% of the level of the wild-type enzyme. Mutations of His181 or equivalent residues in dPGMs from other organisms such as *Schizosaccharomyces pombe* (Nairn et al., 1996) or human erythrocyte bPGM lead to similar loss of activity (Garel et al., 1993). Such mutations are, however, associated with structural alterations which in the case of the yeast enzyme lead to the disruption of its tetrameric structure (White et al., 1993b). Therefore, the unambiguous assignment of the role of this residue in catalysis is still questionable.

The catalytic mechanism is initiated by 3PGA (2PGA) binding to the His8 phosphorylated enzyme's active site (phosphorylated by the 23PGA cofactor), followed by the transfer of the phosphate group from His8 to the C2 (C3) carbon atom of 3PGA (2PGA) to create a reaction intermediate, 23PGA. This substrate phosphorylation part of the reaction is followed by the reorientation of the 23PGA intermediate in the active site to bring the other phosphate group into the proximity of the now available His8 residue. The transfer of this phosphate to this histidine follows in order to regenerate the phospho-enzyme for further catalysis. The 2PGA (3PGA) product then dissociates from the enzyme's active site making it available for the binding of another substrate molecule (Fig 2(b)) (Ridgen et al., 1999).

The mechanism for the other reaction of interconversion of 13PGA and 23PGA have also been proposed by Ridgen et al. (1999). Similar to the main reaction catalyzed by dPGMs, it involves binding of 13PGA to the unphosphorylated enzyme, followed by the transfer of the 1phosphate group to His8 to form a phospho-enzyme intermediate and a 3PGA intermediate. The reorientation of the 3PGA and the transfer of the phosphate to its O2 position winds up the reaction. This review will, however, focus on the main reaction catalyzed by mPGMs (dPGMs and iPGMs) which is the interconversion of 3- and 2PGA.

2.3. Structural comparison of dPGM with other enzymes

The structure of the yeast dPGM enzyme has been found to be similar in sequence and in its three-dimensional structure to two unexpected phosphatase enzymes, fructose-2,6-bisphosphatase (Bazan et al., 1989; Lee et al., 1996; Hasemann et al., 1996) and acid phosphatase (Schneider et al., 1993; LaCount et al., 1998). The sequence similarity of the yeast dPGM (PDB: 1QHF) and the rat liver Fru26P2ase (PDB: 1FBT) is 46% whereas the rat acid phosphatase (AcPase) (PDB: 1RPA) is a bit lower, 35%. Their structures were aligned pairwise with the yeast dPGM enzyme using only the Cα coordinates utilizing the program O (Jones et

al., 1991). There were nine structure-based alignment segments between the yeast dPGM and Fru26P2ase containing 160 residues that aligned with an r.m.s. deviation of 1.63 Å, whereas similar structure-based alignment between the dPGM and the rat AcPase produced six segments containing 95 residues and an r.m.s. deviation of 1.68 Å. The structure-based alignments agreed exactly with the sequence alignment of these enzymes performed using Clustal W (Thompson et al., 1994) (Fig. 1(b)). However, only five residues, Arg7, His8, Asn14, Arg59, and His181, out of all twelve residues (Fig. 1(b), Table 1) implicated in the activity of the yeast dPGM enzyme were conserved in this alignment. These five conserved residues are also conserved among all other PGMs, dPGMs and bPGMs, analyzed in Fig 1(a).

2.3.1. Structural comparison of S. cerevisiae dPGM with 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase

The Fru26P2ase composes only one domain of a bifunctional enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (6PFru-2-K/Fru26P2ase) (EC 2.7.1.105/3.1.3.46) (Lively et

Table 1
Selected essential interactions in the active site of the yeast dPGM enzyme

dPGM residue	Atom type	Substrate analog or protein residue	Atom type	Distance (Å)[a]
Arg7	NH2	3PGA	O1	3.47
	NE	3PGA	O3	3.49
His8	ND1	3PGA	O3	3.64
	NE2	3PGA	O2P	3.83
Gly9	O	His181	ND1	2.84
Ser11	N	3PGA	O1P	3.31
	N	Sulfate1	O3	3.14
	OG	Sulfate1	O4	2.90
Asn14	N	3PGA	O4P	3.81
	ND2	3PGA	O2	3.60
Thr20	OG1	Sulfate1	O4	2.68
Arg59	NE	3PGA	O2P	2.78
Glu86	??	Water	O	??
Arg87	NH2	Sulfate2	O2	3.14
Tyr89	OH	Sulfate2	O1	2.83
Arg113	NE	Sulfate2	O1	2.90
	NH2	Sulfate2	O4	2.93
Arg114	NH1	Sulfate2	O2	2.92
	NH2	Sulfate2	O4	3.14
His181	NE2	His8	NE2	2.79
Asn183	ND2	Sulfate2	O1	3.23

[a] Data based on Crowhurst et al. (1999) for the 3PGA substrate, and on Ridgen et al. (1999) for the complex with sulfate ions as well on the structures from the Protein Data Bank (PDB) accession numbers 1QHF and 5PGM, respectively.

al., 1988) which catalyzes the degradation of fructose-2,6-biphosphate (Fru26P2). The kinase domain catalyzes the synthesis of Fru26P2. Each of the reactions is performed by different active sites of the enzyme located in its two different domains. The regulation of the levels of Fru26P2 is essential for the regulation of glycolysis versus gluconeogenesis through activation or inhibition of 6-phosphofructo-kinase or fructose-1,6-bisphosphatase, two rate-limiting enzymes in sugar metabolism (Bazan et al., 1989; Bazan and Fletterrick, 1990). Such regulation of levels of Fru26P2 leads to the control of the energy storage and levels of blood glucose (Pilkis et al., 1995). Several isozymes of the enzyme have been identified including the enzyme isolated from liver, skeletal muscle, heart, testis, and brain (Darville et al., 1989; Tsuchiya and Uyeda, 1994; Sakata et al., 1991; Ventura et al., 1992). The structure of the dimeric rat liver Fru26P2ase domain was obtained (Lee et al., 1996) followed by the structure of the whole enzyme from the rat testis; also dimeric in nature (Hasemann et al., 1996). Based on the dimeric structure of the whole enzyme, the phosphatase and the kinase domains of the enzyme are well separated and are clearly independent of one another (Hasemann et al., 1996). The kinase domain has an α/β type fold with a central six-stranded β-sheet surrounded by seven α-helices. A nucleotide binding fold is located at the C-terminal end of the first β-strand and this is where the kinase active site is located. Both structures of the phosphatase domain of the whole rat testis enzyme (Hasemann et al., 1996) and the truncated one from rat liver (Lee et al., 1996) are very similar to one another. This phosphatase domain is also similar in structure and sequence to the yeast dPGM enzyme (Fig. 1(b)). Therefore, here we will focus our discussion on the rat liver enzyme's structure of the phosphatase domain alone.

The dimeric phosphatase domain, like other glycolytic enzymes, has an α/β-fold (Sternberg et al., 1981) with the nucleotide-binding topology and the core six-stranded β-sheet surrounded by α-helices on both sides (Lee et al., 1996). The active site of the phosphatase domain is at the C-terminal end of the four-stranded parallel β-sheet. Above the active site there are two long loops that extend away from the core of the enzyme. The area between these loops leads to a funnel-like active site. The active site includes two key histidine residues, His7 and His141, which correspond to the yeast dPGM's His8 and His181 (Bazan et al., 1989; Campbell et al., 1974). A phosphate group has been located in this site in close proximity to the above histidine residues, 4.2 Å away from ND1 His7 and 4.1 Å away from ND1 His141 and the phosphorus atom (Lee et al., 1996). Another residue interacting with the phosphorus atom of the phosphate group is OE1 of Glu76, 3.6 Å away followed by the guanidinium groups of Arg6 (3.7 Å away) and Arg56 (4.0 Å away) (Fig. 3). His7 was also implicated in the formation of the phosphohistidine–enzyme intermediate (Tauler et al., 1990).

2.3.2. Structural comparison of S. cerevisiae dPGM with acid phosphatase

The acid phosphatases, on the other hand, catalyze the hydrolysis of phosphate monoesters and at times a phosphotransfer of phosphate between phosphoester and alcohols. The enzyme catalysis progresses optimally at acidic conditions (Vincent et al., 1992; van Etten, 1982; Bodansky, 1972). These enzymes are found among animals as well as plants (Vincent et al., 1992). In contrast, the totally structurally different alkaline phosphatases (Sowadsky et al., 1985; Kim and Wyckoff, 1991) work optimally at alkaline conditions (Coleman, 1992; Gettins et al., 1985; Coleman and Gettins, 1983; Gettins and Coleman, 1983). The acid phosphatases,

unlike the alkaline phosphatases, do not utilize metal ions in their catalysis. They instead utilize histidine to form an enzyme–phosphohistidine intermediate which is essential for their catalysis (van Etten, 1982). In contrast, alkaline phosphatases utilize a phospho-serine enzyme intermediate for their catalysis and have a binuclear Zn(II) active site (Coleman, 1992; Coleman et al., 1983, 1997) and are related to the iPGM enzymes (Galperin et al., 1995; Jedrzejas et al., 2000a; Jedrzejas and Setlow, 2000).

The structure of rat prostatic acid phosphatase, which is also dimeric in nature, was determined by Schneider et al. (1993) followed by the structure of the human prostatic enzyme determined by LaCount et al. (1998). The structure of the enzyme comprises two domains, an α/β type domain and a smaller α-helical domain (Schneider et al., 1993). The α/β domain has a core of seven-stranded mixed β-sheets flanked on both sides by α-helices. The α-domain forms a flat disk-like cap above the other domain of the enzyme. The active site is located in an open, easily accessible cleft between the two domains at the C-terminal end of the parallel β-strands of the α/β domain. The residues conserved among acid phosphatases that are in the active site area are implicated in the activity of this enzyme and are as follows: Arg11, His12, Arg15, Arg79, His257, and Asp258 (Fig. 4) (Schneider et al., 1993). His12 has been proposed to form a phosphohistidine–enzyme intermediate (Ostanin et al., 1992). This residue is in close proximity to several Arg residues: Arg11, Arg15, Arg79 and His257, which create a positively charged pocket suggestive of phosphate binding. His12 and His257 correspond to similar residues in the dPGM enzyme, His8 and His181, respectively (Fig. 1(b)). All of these residues, except Arg15, are conserved in sequence and structure among all three enzymes discussed here and are conserved among all dPGM enzymes' sequences (Fig. 1(a) and (b).

2.3.3. Overall comparison of active sites of dPGM, Fru26P2ase, and AcPase

Based on an analysis of structural data and mutagenesis results together with the sequence analysis of all known dPGM enzymes, 14 residues were implicated in the catalysis carried out by dPGMs (Table 1). Out of these 14 residues only six are uniformly conserved among Fru26P2ases and AcPase enzymes: Arg7, His8, Glu9, Asn14, Arg59, and His181 (Figs. 1(a) and (b), 3 and 4). The precise mechanism of the dPGM catalytic process is still largely speculative. However, a crucial component, a histidine residue that accepts a phosphate group during the reaction, has been unequivocally identified (Rose, 1971; Nairn et al., 1995). Another histidine residue, His181, was confirmed as being very important for catalysis but its precise function is still speculative (White and Fothergill-Gilmore, 1992; White et al., 1993a). Originally, this histidine was suggested to perform an acid/base type of function based on the donation or extraction of a proton during the formation of phosphohistidine intermediate (donation of H for neutralization of the O_2 (O_3) oxygen of the substrate's glycerate moiety) or during the hydrolysis of the phosphohistidine intermediate (extraction of H from a water molecule hydrolyzes the His8–phosphate bond) (Rose, 1980). This function could also be performed, as recently suggested by Ridgen et al. (1999), by a water molecule polarized by Glu86. This scenario is consistent with studies of the E86Q mutant of the yeast enzyme, which retains less than 5% of the wild-type enzyme activity (Ridgen et al., 1999). Surprisingly, Glu86 is not conserved in the human or in the rat prostatic AcPases. It is conserved, however, in the rat liver and testis Fru26P2ase (Glu76) (Figs. 1(b), 3 and 4) (Lee et al., 1996; Hasemann et al.,

1996). An inspection of the prostatic AcPase enzyme structure, however, shows Asp258 in a nearby location. This residue could interact with the proposed catalytic water molecule and facilitate the mechanism as would a Glu residue. The precise mechanism leading to the formation and subsequent hydrolysis of the enzyme-phosphate complex cannot be unquestionably delineated at this time. This is largely due to the complexity of the mechanism and the flexible nature of at least part of the residues in the active site including the C-terminal tail capping this site. The structural information on sulfate (Ridgen et al., 1999) and especially on the 3PGA substrate binding in the active site (Crowhurst et al., 1999) clarified significantly the proposed catalytic mechanism of this enzyme. The structure of these complexes definitely narrowed down the possible mechanism involved in the catalysis and pointed to some new residues or to the assignment of different functions for some of them. For example, for the yeast dPGM the proposed function of His181 as a proton donor/acceptor was later replaced by a proposed functional water molecule and a close-by Glu86 residue (Ridgen et al., 1999). However, more structural information on substrate/product binding in the active site would help identify all residues involved in the catalysis and contribute to the determination of their precise role. Such information will likely be obtained in the future.

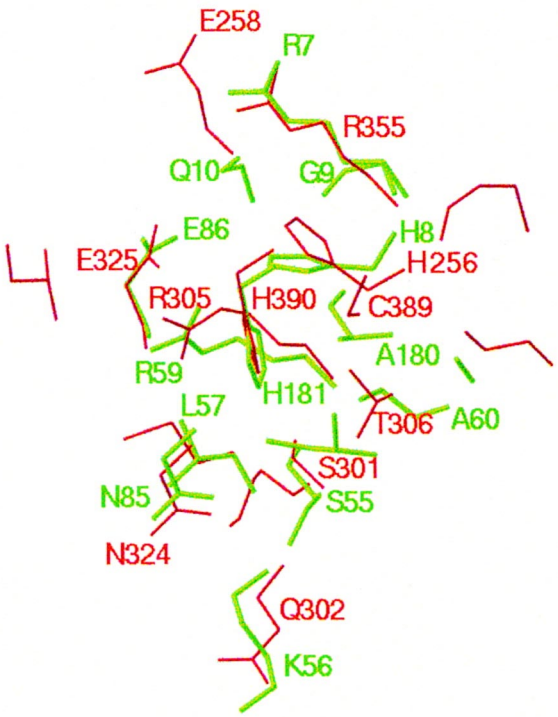

Fig. 3. The residues in the active site area of the *S. cerevisiae* dPGM (PDB: 1QHF) aligned with the rat liver fructose-2,6-bisphosphatase (Lee et al., 1996) (PDB: 1FBT). Among other conserved residues, both catalytic histidines, His8/His7 and His181/His141, are conserved.

Based on the sequence analysis (Fig. 1(b)) and the overall structure similarities as well as the similarities of the active sites of the three enzymes, 23PGA-dependent monophosphoglycerate mutase, fructose-2,6-bisphosphatase, and acid phosphatase are very similar in their properties. The catalytic mechanism of these enzymes seems to follow the same paradigm and consists of two main steps: phosphorylation of a histidine residue to create an enzyme-phosphate intermediate and subsequent hydrolysis of this phosphate to transfer this group to the substrate or to release an inorganic phosphate group. The first possibility corresponds to the function of dPGM and AcPase enzymes and has two aspects, a phosphatase and a phosphotransferase activity, the second to the Fru26P2ase which has only the phosphatase part of the reaction. As stated above, the AcPase enzymes have the ability to phosphorylate themselves (phosphatase activity), to release this phosphate group by the hydrolysis of the phosphate monoester linkage to the enzyme (part of the phosphatase activity), or to transfer the phosphate to other chemical groups such as alcohols (phosphotransferase ability). All three enzymes have the ability, however, to perform all these reactions albeit with very different catalytic rates and all these reactions involve the same phosphohistidine intermediate (Tauler et al., 1987).

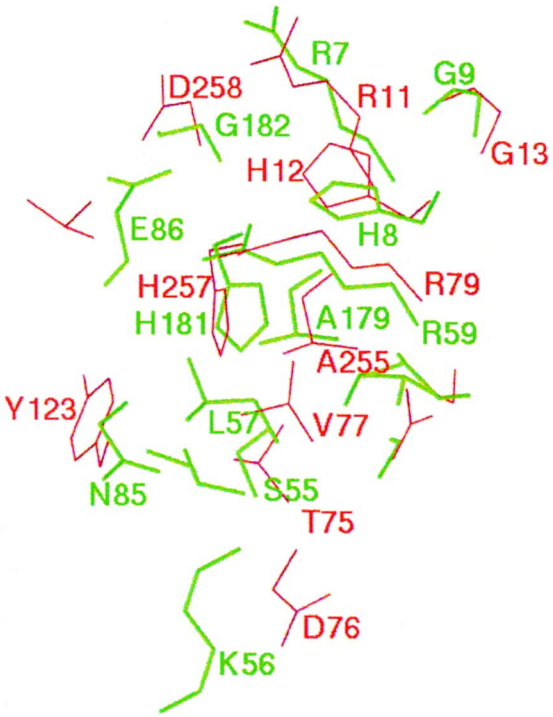

Fig. 4. Active site residues of *S. cerevisiae* dPGM aligned with those of the rat prostatic acid phosphatase (Schneider et al., 1993) (PDB: 2RPA). Both active site histidine residues, His8/His12 and His181/His257, are also conserved.

For dPGM, Fru26P2ase, and AcPase the presence of the phosphohistidine intermediate has been confirmed and localized, in each case, to one His residue: His8 for the yeast dPGM, His7 for the human Fru26P2ase, and His12 for the rat prostatic AcPase enzyme. Moreover, for all these enzymes other histidine residues, His181 for the yeast dPGM, His141 for Fru26P2ase, and His257 for AcPase, were identified to be very important for catalysis. Mutation of this histidine leads to very significantly compromised enzymes. Both histidines are conserved in the sequence analysis shown in Fig. 1(a) and (b) as well as in the three dimensional structures shown in Figs. 3 and 4. Similar to the yeast dPGM, all residues involved in the activity related to each of these above enzymes have not, however, been clearly identified, in part due to the expected flexibility of these active sites and the immediate adjacent areas of the structure. In addition, even though the general aspects of the catalytic mechanism of these enzymes have been known for many years, the exact mechanisms and involved residues are still unknown. Significant hints were provided by the structural information of complexes of these enzymes with substrate or substrate mimicking chemicals such as the recent structural data on the yeast dPGM with 3PGA or sulfate residues in its active sites (Ridgen et al., 1999; Crowhurst et al., 1999).

The functional and structural similarities of all three enzymes analyzed above provide strong evidence for their close evolutionary similarity and, furthermore, show that they are evolutionarily related (see also Bazan et al., 1989). These enzymes catalyzing similar reaction types are likely to have descended from a common ancestral protein and acquired their unique features through divergent evolution (Fothergill-Gilmore, 1987; Rossmann, 1981). The divergent sequences and different preferred substrates suggest that the progenitor of all these different enzymes possibly had the affinity for a negatively charged molecule such as a small molecule or an ion, with a nucleotide-like binding fold built from β-sheets and α-helices. The definitively defined active site possibly developed later with one or two catalytic groups like the histidine residues, His8 and His181 (dPGM numbering scheme) (Bazan and Fletterrick, 1990). During the course of evolution, the initial/progenitor enzyme developed differences in its active site and its adjacent areas, loops or other structures, evident from the comparison of the structures of the three enzymes discussed above, and based on surface features that probably led to the specialization of function and the emergence of three different albeit similar families of enzymes and dPGMs together with the closely related bPGMs, Fru26P2ases, and AcPases (Fig. 1(a) and (b)) (Bazan and Fletterrick, 1990; Tauler et al., 1987). The relationship between these enzymes is not obvious from their primary structures. However, the comparison of their three-dimensional structures finally revealed their similarities. As is often the case, the tertiary structure seems to be more conserved than the primary structure (Chothia et al., 1987; Richardson, 1981).

As mentioned earlier, the dPGM and bPGM enzymes have the ability to catalyze the same reactions but at significantly different rates. dPGM catalyzes the 23PGA-dependent interconversion of 2- and 3PGA, and bPGM catalyzes the interconversion of 13PGA and 23PGA (Fothergill-Gilmore and Watson, 1989). In addition to performing similar catalytic functions, significant sequence homology of these two group of enzymes supports the notion that these two groups of enzymes are isoenzymes. The sequence similarities among the dPGMs are, however, higher than their sequence similarity with the bPGM enzyme (Fig. 1(a)). This is

suggestive of gene divergence being responsible for the creation of bPGMs followed later by the evolutionary divergence among dPGMs (Fothergill-Gilmore, 1989).

It seems, however, that the other type of PGMs, the 23PGA independent mPGM (iPGM), is not evolutionarily related to any of the other PGMs, or to the other enzymes related to dPGM/bPGM, Fru26P2ases or AcPases (Fothergill-Gilmore and Watson, 1989). Having in mind that the iPGMs are approximately twice as large as the dPGM/bPGM enzymes, it is possible that the development of the iPGM enzymes might have been a gene-doubling process as has been observed for a number of other enzymes (Lawrence and Trayer, 1984; Poorman et al., 1984). The three-dimensional structural information about the iPGM enzyme from *Bacillus stearothermophilus*, representative of all iPGM enzymes, have only recently been made available (Jedrzejas et al., 2000a). The structure of this iPGM has two distinct domains which either individually or together do not structurally align in any way with the yeast dPGM enzyme structure. Sequence comparison of both types of PGMs, combined with their lack of any three-dimensional structural alignment/similarity, of these two enzymes clearly shows that these enzymes did not evolve through the gene-doubling process. Significant structural homology has been, however, identified between the iPGM enzyme and the *E. coli* alkaline phosphatase (AlPase) (Jedrzejas and Setlow, 2000; Jedrzejas et al., 2000a). The distribution of these two types of PGMs in different organisms, dPGMs specific to vertebrates and iPGM specific to plants (see Section 1) (Carreras et al., 1982; Price et al., 1985), suggests that both types of genes were present early in evolution. Animals lost the iPGM gene prior to the radiation of vertebrates whereas plants probably lost the dPGM gene when the early forms diverged from the primitive unicellular organisms (Fothergill-Gilmore and Watson, 1989).

Some eubacteria with larger genomes such as *E. coli* or *B. subtilis*, however, have both dPGM and iPGM genes and both of these genes are expressed at somewhat similar levels (Fraser et al., 1999; Kunst et al., 1997). Also, in *E. coli* both of these enzymes, dPGM and iPGM, were active albeit at somewhat different rates (Fraser et al., 1999). In general the dPGM enzyme appears to be more active in *E. coli*. In their evolution, these selected unicellular eubacterial organisms somehow managed to retain both genes.

3. *Bacillus stearothermophilus* cofactor independent phosphoglycerate mutase

3.1. Structure of B. stearothermophilus phosphoglycerate mutase

The structure of the first iPGM enzyme has just recently been published by Jedrzejas et al. (2000a). The *B. stearothermophilus* iPGM enzyme consists of 511 residues (57 kDa) and its active form structure contains two Mn(II) ions (Chander et al., 1999). It is a monomer which contains two distinctly separated domains of approximately equal size which interact with one another through an extended surface area contact (Fig. 5(a)). There is a narrow cleft between these domains which is where the active site of the enzyme is located. This crystal structure also includes a 3PGA substrate as well as a catalytic, ordered water molecule bound in the active site of the enzyme (Fig. 5(b)). Both domains have a central β-sheet surrounded by α-helices on the outside. The active site contains residues from both domains. However, residues interacting with the Mn(II) ions and the phosphate group of 3PGA originate from one

domain, A, whereas residues interacting with the glycerate part of the substrate originate from the other domain, B.

3.2. Catalytic mechanism for B. stearothermophilus iPGM and its comparison to alkaline phosphatase catalysis

The precise mechanism of catalysis of this iPGM was proposed based on the complex structure with 3PGA and Mn(II), structure-guided mutant studies, sequence and structure comparison with the *E. coli* AlPase (Jedrzejas et al., 2000a, 2000b), and earlier biochemical studies (Smith et al., 1988; Leadlay et al., 1977). Another structure was recently obtained for the iPGM complex with the other substrate/product, 2PGA, which closes the catalytic loop of a substrate to a product transformation or vice versa (Jedrzejas et al., 2000b). The catalytic mechanism from 3- to 2PGA isomerisation involves phosphorylation of the Ser62 residue by 3PGA (phosphatase activity) followed by the reorientation of the remaining glycerate of 3PGA and the transfer of the phosphate to the O2 oxygen of this repositioned glycerate (phosphotransferase activity). The active site water molecule hydrolyzes the interaction of the 2PGA product with Mn(II) ion and frees up the active site for the next round of catalysis. Both Mn(II) ions are inherently involved in this catalytic process (Dismukes, 1996) through their interactions with active site residues and the substrates/products as well as the ordered active site water molecule.

For spore forming bacteria, such as *B. stearothermophilus*, a Mn(II) requirement leads to a physiologically relevant pH sensitivity of the enzyme (Kuhn et al., 1991; Kuhn et al., 1993, 1995). A pH of 8 is optimal for enzyme activity whereas activity is essentially lost at pH ~6. In spores the pH is as low as 6.5 in comparison to the one in the vegetative cells which is ~8.0 (Chander et al., 1998; Swerdlow et al., 1981; Singh and Setlow, 1979). Therefore, during the sporulation process pH drops down to ~6.5 and iPGM becomes inactive allowing for 3PGA deposits that can be later utilized as the main source of energy required for the germinating spore when pH rises back to ~8. The new vegetative cell utilizes the 3PGA deposits in the glycolysis pathway to form ATP necessary for other biochemical reactions (Magil et al., 1994, 1996; Setlow and Kornberg, 1970). For a detailed description of the iPGM mechanism please refer to Jedrzejas et al. (2000a, 2000b) as well as to Jedrzejas and Setlow (2000).

The information shown above allowed the assignment of the phosphatase activity of the enzyme to domain A and domain B to the phosphotransferase activity. The phosphatase domain of iPGM has been shown to have a general fold similar to *E. coli* AlPase (Sowadski et al., 1985). Furthermore, the structural positioning of all metal-binding residues in the active sites for both enzymes is also essentially identical (Fig. 6). However, there is no similarity between the phosphotransferase domain of iPGM and the AlPase.

A detailed comparison of both enzyme types, iPGMs and AlPases, has also been provided based on their sequences and three-dimensional structures, by Jedrzejas and Setlow (2000), and based on a sequence in an earlier report by Galperin et al. (1998). Both the phosphatase domain of iPGM and the AlPase enzyme were clearly identified to be structurally and functionally related to one another. Both are binuclear metalloenzymes (Dismukes, 1996; Johnson and Price, 1988), the *B. stearothermophilus* being Mn(II)-dependent whereas *E. coli* AlPase is Zn(II)-dependent (Applebury et al., 1970; Anderson et al., 1975; Applebury and

Coleman, 1969; Bosron et al., 1975, 1977), and their similar catalytic mechanisms utilize a phosphoserine–enzyme intermediate. This mechanism is unlike the behavior of dPGMs/Fru26P2ases/AcPases which utilize a phosphohistidine–enzyme intermediate for their catalysis.

As noted earlier, iPGMs are specific to plants as well as some other organisms/cells (see Section 1) whereas AlPases have been identified in animal, yeast, and bacterial cells but, not so far, in plants (Galperin et al., 1998). Considering the structural, functional, and mechanistic similarities of both iPGMs and AlPases (similar to the dPGMs/Fru26P2ases/AcPases enzymes), it is possible that these two groups of proteins (iPGMs and AlPases) are evolutionarily related and have evolved from a common ancestor enzyme; different from the dPGM related ancestral enzyme discussed earlier. Furthermore, similarities have already been identified (Galperin et al.,

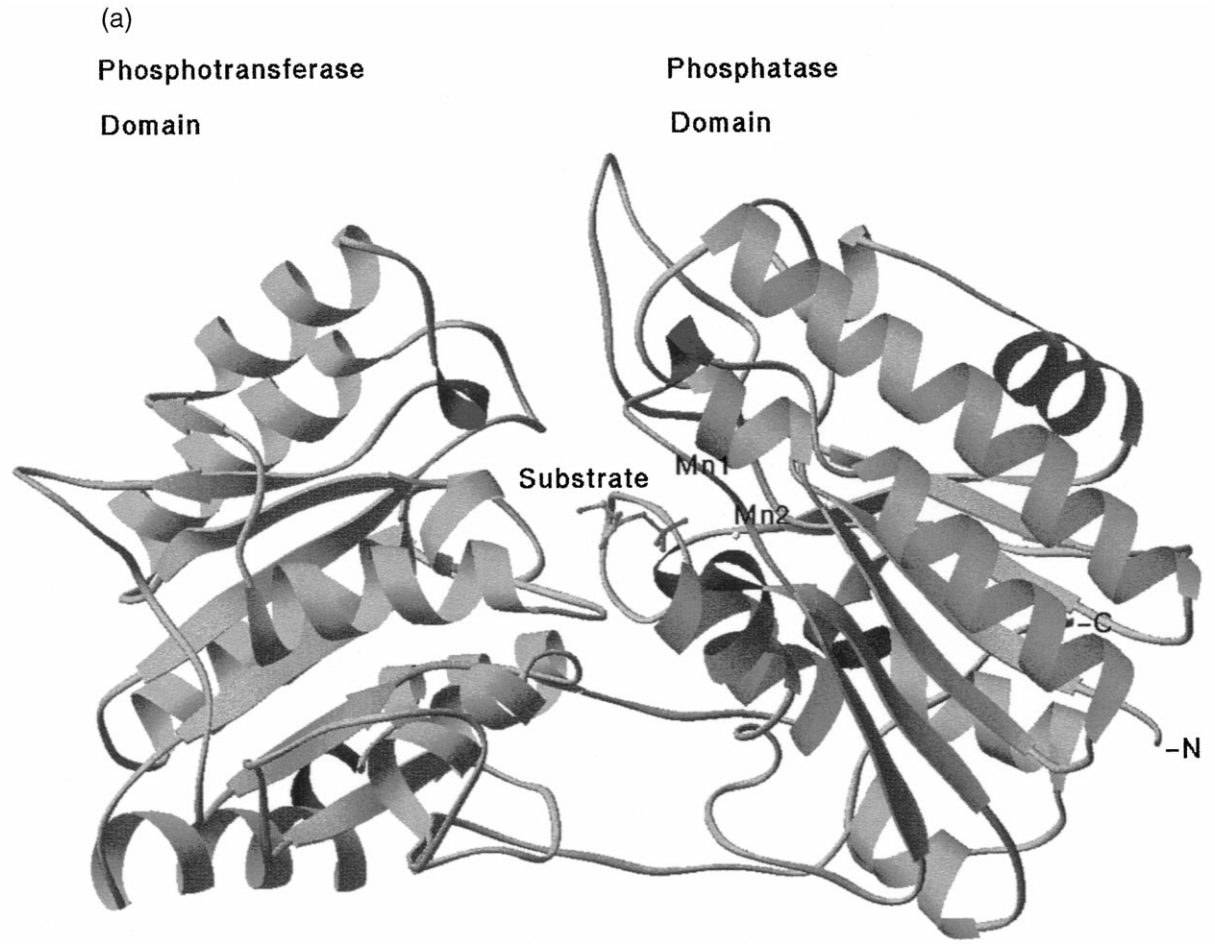

Fig. 5. (a) General structure of *B. stearothermophilus* iPGM enzyme. Both the phosphatase and the phosphatransferase domains are labeled along with the active site containing 3PGA substrate/product (Jedrzejas et al., 2000a). (b) Active site residues color coded by the domain: phosphatase domain B — green, phosphotransferase domain A — red. Two Mn(II) ions as well as the catalytic water and 3PGA are also shown.

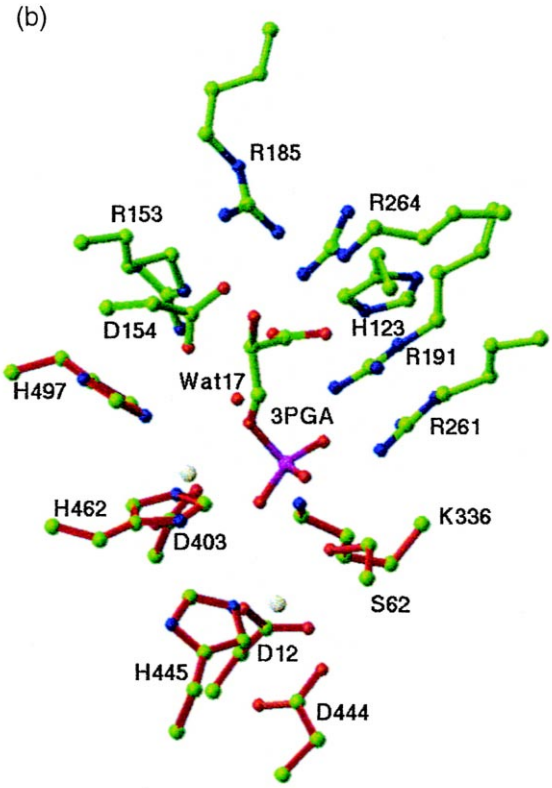

Fig. 5 (*continued*)

1998) between all known iPGMs and AlPases among archeal proteins such as *Methanococcus jannaschii* MJ1612 and MJ0010 (Koonin et al., 1997) and paralogous proteins in *Methanobacterium thermoautotrophicum* (Smith et al., 1997) or in *Archaeoglobus fulgidus* (Klenk et al., 1997).

4. Functional properties and comparison of the *S. cerevisiae* dPGM with the *B. stearothermophilus* iPGM enzyme

Both enzyme groups, dPGMs and iPGMs, perform their catalysis through a phosphoenzyme intermediate. For the cofactor dependent dPGM enzyme, the enzyme is probably phosphorylated by the 23PGA cofactor on His8, a process required for the enzyme activity. The functional, active enzyme form is its phosphorylated enzyme. For the cofactor independent iPGM enzyme, the substrate itself phosphorylates the enzyme not on a histidine but on a Ser62 residue. The native and active iPGM enzyme is not a phospho–enzyme. Although the dPGM enzymes are not metalloenzymes, all iPGM enzymes are metalloenzymes and most likely all

utilize manganese ions as do the *B. stearothermophilus* and *E. coli* enzymes (Jedrzejas and Setlow, 2000). However, the substitution of Mn(II) for other metals, especially Zn(II), cannot be totally excluded at this time. The pH sensitivity of iPGMs from spore-forming bacteria is physiologically relevant but for other bacterial organisms such as *E. coli* it is not. Therefore, it seems likely that all bacterial iPGM enzymes evolved from a gene in a common bacterial ancestor having the ability to create spores which later in the evolutionary process some of these organisms lost.

For both enzymes, after the phosphorylation step a significant reaction intermediate reorientation must occur to either remove a phosphate from the reaction intermediate for dPGM or to transfer the phosphate to the appropriate place in the glycerate for iPGM. None of the reactions of iPGM catalysis, except for the final release of the product, involve a hydrolysis step due to the metalloenzyme nature of iPGM, and the ability of Mn(II) to neutralize charge through its interactions (Christianson and Cox, 1999; Christianson, 1997). For dPGM, however, two extra hydrolysis steps are likely required: hydrolysis of the phospho–enzyme bond and the hydrolysis of the reaction intermediate–phosphate bond. Implicated in

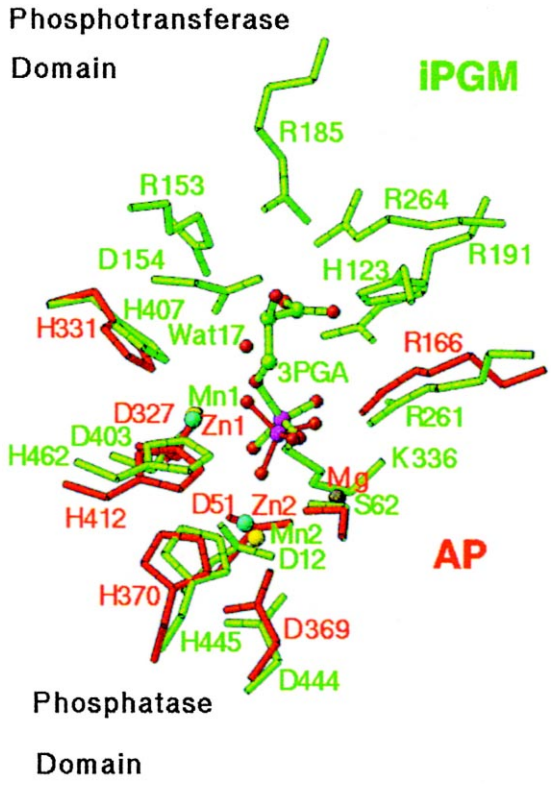

Fig. 6. Active site alignment of the phosphatase domain of *B. stearothermophilus* iPGM and *E. coli* AlPase. Both Mn(II) ions, catalytic water and 3PGA of iPGM and two Zn(II) ions of AlPase are also shown.

this process was the role of the acid/base hydrolysis of His181 or activation of a water molecule by Glu86. Since the mutants of either His181 or Glu86 still retain some enzyme activity, it is possible that both mechanisms (acid/bone hydrolysis and water activation) can perform this necessary function for catalysis. The dPGM enzyme as well as bPGM have an additional ability to interconvert 13PGA and 23PGA as well as synthesize the 3PGA from 23PGA. The iPGM enzyme is limited in this aspect as it only interconverts 3- and 2PGA.

5. Conclusions

Clearly, both dPGM and bPGM enzymes are evolutionarily related. This relation is visible in both their primary and tertiary structures. Their catalysis proceeds via a phosphohistidine–enzyme intermediate and also involves the reorientation of their substrates in the process. These two groups of related enzymes show a moderate to low sequence homology to Fru26P2ases and to AcPases but the comparison of the tertiary structures of all these enzymes more clearly shows their structural and evolutionary similarity. All of these enzymes phosphorylate a histidine residue, a process essential for their catalytic activity which requires two main components, phosphatase and phosphotransferase. Some of these enzymes specialize in just one component, the phosphatase reaction, but all have the ability to perform both of them albeit at different rates. All of these enzymes are evolutionarily related to a common ancestral enzyme and they probably acquired their specialized traits through divergent evolution, although gene duplication can not be excluded. The divergence of the protein surface, the gain of loops, as well as changes in the areas adjacent to the active site presumably resulted in the catalytic differences leading to different enzymes: PGMs, Fru26P2ases, and AcPases.

The iPGM enzymes, on the other hand, are not related to any of the other PGM group of enzymes, both in sequence and in three-dimensional structure, but are related to the family of alkaline phosphatases. This relationship is hardly evident from the primary structures of iPGMs and AlPases (unlike the relationship for dPGMs/bPGMs/Fru26P2ases/AcPases) whereas clearly evident from the comparison of the tertiary structures, predominantly for the active site residues responsible for metal binding. In terms of evolution, iPGMs and AlPases probably evolved from a common predecessor, an archeal protein. The specialization of the functional properties of the current enzymes probably evolved similarly to the dPGM family through changes of the active site environment and adjacent loop areas, as well as through divergence of protein surfaces.

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References

Anderson, R.A., Bosron, W.F., Kenned, F.S., Vallee, B.L., 1975. Role of magnesium in *Escherichia coli* alkaline phosphatase. Proc. Natl. Acad. Sci. USA 72, 2989–2993.

Applebury, M.L., Coleman, J.E., 1969. *Escherichia coli* Co(II) alkaline phsophatase. J. Biol. Chem. 244, 709–718.

Applebury, M.L., Johnson, B.P., Coleman, J.E., 1970. Phosphate binding to alkaline phosphatase. Metal ion dependence. J. Biol. Chem. 245, 4968–4976.

Bazan, J.F., Fletterrick, R.J., 1990. In: Pilkis, S.J. (Ed.), Fructose 2,6-Bisphosphate. CRC Press, Boca Raton, FL, pp. 125–171.

Bazan, J.F., Fletterrick, R.J., Pilkis, S.J., 1989. Evolution of a bifunctional enzyme: 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase. Proc. Natl. Acad. Sci., USA 86.

Blanchet, C., Geourjon, C., Deleage, G., 1999. Multiple Protein Sequence Analysis. IBCP, CNRS UPR 412, L.on, France.

Blattler, A.W., Knowles, J.R., 1980. Phosphoglycerate mutases: Stereochemical course of the phosphoryl group transfers catalyzed by the cofactor-dependent enzyme from rabbit muscle and the cofactor-independent enzyme from wheat germ. Biochemistry 19, 738–743.

Bodansky, O., 1972. Acid phosphatase. Adv. Clin. Chem. 15, 43–147.

Bosron, W.F., Anderson, R.A., Falk, M.C., Kennedy, F.S., Vallee, B.L., 1977. Effect of magnesium on the properties of zinc alkaline phosphatase. Biochemistry 16, 610–614.

Bosron, W.F., Kennedy, F.S., Vallee, B.L., 1975. Zinc and magnesium content of alkaline phosphatase from *Escherichia coli*. Biochemistry 14, 2275–2282.

Breathnach, R., Knowles, J.R., 1977. Phosphoglycerate mutase from wheat germ: studies with ^{18}O-labeled substrate, investigations of the phosphatase and phosphoryl transfer activities, and evidence for a phosphoryl-enzyme intermediate. Biochemistry 16 (14), 3054–3060.

Britton, H.G., Carreras, J., Grisolia, S., 1971. Mechanism of action of 2,3-diphosphoglycerate-independent phosphoglycerate mutase. Biochemistry 11, 4522–4533.

Campbell, J.W., Watson, H.C., Hodgson, G.I., 1974. Structure of yeast phosphoglycerate mutase. Nature 250, 301–303.

Carreras, J., Mezquita, J., Bosch, J., Bartrons, R., Pons, G., 1982. Phylogeny, ontogeny of the phosphoglycerate mutases-IV. Distribution of glycerate-2,3-P2 dependent and independent phosphoglycerate mutases in algae, fungi, plants and animals. Comp. Biochem. Physiol. [B] 71, 591–597.

Chander, M., Setlow, B., Setlow, P., 1998. 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.

Chander, M., Setlow, P., Lamani, E., Jedrzejas, M.J., 1999. Structural studies on a 2,3-diphosphoglycerate independent phosphoglycerate mutase from Bacillus stearothermophilus. J. Struct. Biol. 126, 156–165.

Chothia, C., Lesk, A.M., 1987. The evolution of protein structures. Cold Spring Harb. Symp. Quant. Biol. 52, 399–405.

Christianson, D.W., Cox, J.D., 1999. Catalysis by metal-activated hydroxide in zinc and manganese metalloenzymes. Annu. Rev. Biochem. 68, 35–57.

Christianson, D.W., 1997. Structural chemistry and biology of manganese metalloenzymes. Prog. Biophys. Molec. Biol. 67, 217–252.

Coleman, J.E., 1987. In: Torriani-Gorini, A., Rothman, F.G., Silver, S., Wright, A., Yagil, E. (Eds.), Phosphate Metabolism and Cellular Regulation in Microorganisms. Am. Soc. Microbiol, Washington, DC, pp. 127–138.

Coleman, J.E., 1992. Structure and mechanism of alkaline phosphatase. Annu. Rev. Biophys. Biomol. Struc. 21, 441–483.

Coleman, J.E., Gettins, P., 1983. Alkaline phosphatase, solution structure, and mechanism. Adv. Enzymol. Relat. Areas Mol. Biol. 55, 381–452.

Coleman, J.E., Nakamura, K., Chlebowski, J.F., 1983. ^{65}Zn(II), ^{115}Cd(II), ^{60}Co(II), and Mg(II) binding to alkaline phosphatase of *Escherichia coli*. Structural and functional effects. J. Biol. Chem. 258, 386–395.

Crowhurst, G.S., Dalby, A.R., Isupov, M.N., Campbell, J.W., Littlechild, J.A., 1999. Structure of a phosphoglycerate mutase: 3-phosphoglyceric acid complex at 1.7 Å. Acta Crystallog. Sect. D. 55, 1822–1826.

Darville, M.I., Crepin, K.M., Hue, L., Rousseau, G.G., 1989. 5′ flanking sequence and structure of a gene encoding rat 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase. Proc. Natl. Acad. Sci., USA 86, 6543–6547.

Dismukes, G.C., 1996. Manganese enzymes with binuclear active sites. Chem. Rev. 96, 2909–2926.

Fothergill-Gilmore, L.A., 1987. Evolution in glycolysis. Biochem. Soc. Trans. 15, 993–995.

Fothergill-Gilmore, L.A., Michels, P.A.M., 1993. Evolution of glycolysis. Progr. Biophys. Mol. Biol. 59, 105–235.

Fothergill-Gilmore, L.A., Watson, H.C., 1989. The phosphoglycerate mutases. Adv. Enzymol. 62, 227–313.

Fraser, H.I., Kratskhelia, M., White, M.F., 1999. The two analogous phosphoglycerate mutases of *Escherichia coli*. FEBS Lett. 455, 344–348.

Galperin, M.Y., Bairoch, A., Koonin, E.V., 1998. A superfamily of metalloenzymes unifies phosphopentomutase and cofactor-independent phosphoglycerate mutase with alkaline phosphatases and sulfatases. Prot. Science 7, 1829–1838.

Garel, M.C., Joulin, V., Le Boulch, P., Calvin, M.C., Prehu, M.O., Arous, N., Longin, R., Rosa, R., Rosa, J., Cohen-Solal, M., 1989. Human bisphosphoglycerate mutase. Expression in *Escherichia coli* and use of site-directed mutagenesis in the evaluation of the role of the carboxyl-terminal region in the enzymatic mechanism. J. Biol. Chem. 264, 18966–18972.

Garel, M.C., Lemarchandel, V., Calvin, M.C., Arous, N., Craescu, C.T., Prehu, M.O., Rosa, J., Rosa, R., 1993. Amino acid residues involved in the catalytic site of human erythrocyte bisphosphoglycerate mutase. Functional consequences of substitutions of His187 and Arg89. Eur. J. Biochem. 213, 493–500.

Gatehouse, J.A., Knowles, J.R., 1977. Phosphoglycerate mutase from wheat germ: studies with isotopically labeled 3-phospho-D-glycerates showing that the catalyzed reaction is intramolecular. Biochemistry 16, 3045–3050.

Gettins, P., Coleman, J.E., 1983. 31P nuclear magnetic resonance of phosphoenzyme intermediates of alkaline phosphatase. J. Biol. Chem. 258, 408–416.

Gettins, P., Metzler, M., Coleman, J.E., 1985. Alkaline phosphatase. 31P NMR probes of the mechanism. J. Biol. Chem. 260, 2875–2883.

Grana, X., Lecea, L., El-Maghrabi, M.R., Urena, J.M., Caellas, C., Carreras, J., Puigdomenech, P., Pilkis, S.J., Climent, F., 1992. Cloning and sequencing of a cDNA encoding 2,3-bisphosphoglycerate-independent phosphoglycerate mutase from maize. J. Biol. Chem. 267, 12797–12803.

Grana, X., Perez De La Ossa, P., Broceno, C., Stocker, M., Garriage, J., Puigdomenech, P., Climent, F., 1995. 2,3-Bisphosphoglycerate-independent phosphoglycerate mutase is conserved among different phylogenetic kingdoms. Comp. Biochem. Physiol. 112B, 287–293.

Han, C-H., Rose, Z.B., 1979. Active site phosphohistidine peptides from red cell bisphosphoglycerate synthase and yeast phosphoglycerate mutase. J. Biol. Chem. 254, 8836–8839.

Hasemann, C.A., Istvan, E.S., Uyeda, K., Deisenhofer, J., 1996. The crystal structure of the bifunctional enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase reveals distinct domain homologies. Structure 4, 1017–1029.

Jedrzejas, M.J., Chander, M., Setlow, P., Krishnasamy, G., 2000a. Crystal structure and mechanism of action of 2,3-diphosphoglycerate independent phosphoglycerate mutase from *Bacillus stearothermophilus*. EMBO J. 19, 1419–1431.

Jedrzejas, M.J., Chander, M., Setlow, P., Krishnasamy, G., 2000b. Mechanism of catalysis of the cofactor independent phosphoglycerate mutase from *Bacillus stearothermophilus*: crystal structure of the complex with 2-phosphoglycerate. J. Biol. Chem., in press.

Jedrzejas, M.J., Setlow, P., 2000. Comparison of the binuclear metalloenzymes diphosphoglycerate-independent phosphoglycerate mutase and alkaline phosphatase: their mechanism of catalysis via a phosphoserine intermediate. Chem. Rev., submitted.

Johnson, M., Price, N.C., 1988. Do metal ions promote the re-activation of the 2,3-bisphosphoglycerate-independent phosphoglycerate mutases? Biochem. J. 252, 111–117.

Jones, S.R., Kidman, L.A., Knowles, J.R., 1978. Stereochemistry of phosphoryl group transfer using a chiral [^{16}O, ^{17}O, ^{18}O] stereochemical course of alkaline phosphotase. Nature 275, 564–565.

Jones, T.A., Zou, J-T., Cowan, S.W., Kjeldgaard, M., 1991. Improved methods for building protein models in electron density maps and the location of errors in these maps. Acta Cryst. A47, 110–119.

Kim, E.E., Wyckoff, H.W., 1991. Reaction mechanism of alkaline phosphatase based on crystal structures. J. Mol. Biol. 218, 449–464.

Klenk, H.P., Clayton, R.A., Tomb, J.F., White, O., Nelson, K.E., Ketchum, K.A., Dodson, R.J., Gwinn, M.,

Hickey, E.K., Peterson, J.D., Richardson, D.L., Kerlavage, A.R., Graham, D.E., Kyrpides, N.C., Fleischmann, R.D., Quackenbush, J., Lee, N.H., Sutton, G.G., Gill, S., Kirkness, E.F., Dougherty, B.A., McKenn, K., Adams, M.D., Loftus, B., Venter, J.C., et al., 1997. The complete genome sequence of the hyperthermophilic, sulphate-reducing archaeon *Archaeoglobus fulgidus*. Nature 390, 364–370.

Koonin, E.V., Mushegian, A.R., Galperin, M.Y., Walker, D.R., 1997. Comparison of archaeal and bacterial genomes: computer analysis of protein sequences predicts novel functions and suggests a chimeric origin for the archaea. Mol. Microbiol. 25, 619–637.

Kuhn, N.J., Setlow, B., Setlow, P., 1993. Manganese(II) activation of 3-phosphoglycerate mutase of *Bacillus megaterium*: pH-sensitive interconversion of active and inactive forms. Arch. Biochem. Biophys. 306, 342–349.

Kuhn, N.J., Setlow, B., Setlow, P., Cammack, R., Williams, R., 1995. Cooperative manganese(II) activation of 3-phosphoglycerate mutase of *Bacillus megaterium*: a biological pH-sensing mechanism in bacterial spore formation and germination. Arch. Biochem. Biophys. 319, 35–42.

Kunst, F., Ogasawara, N., Moszer, I., Albertini, A.M., Alloni, G., Azevedo, V., Bertero, M.G., Bessieres, P., Bolotin, A., Borchert, S., Borriss, R., Boursier, L., Brans, A., Braun, M., Brignell, S.C., Bron, S., Brouillet, S., Bruschi, C.V., Caldwell, B., Capuano, V., Carter, N.M., Choi, S.K., Codani, J.J., Connerton, I.F., Danchin, A., et al., 1997. The complete genome sequence of the gram-positive bacterium *Bacillus subtilis*. Nature 390, 249–256.

LaCount, M.W., Handy, G., Lebioda, L., 1998. Structural origins of L(+)-tartrate inhibition of human prostatic acid phosphatase. J. Biol. Chem. 273, 30406–30409.

Lawrence, GM., Trayer, I.P., 1984. Hexokinase isoenzymes: antigenic cross-reactivities and amino acid compositional relatedness. Comp. Biochem. Physiol. B 79, 233–238.

Leadlay, P.F., Breathnach, R., Gatehouse, J.A., Johnson, P.E., Knowles, J.R., 1977. Phosphoglycerate mutase from wheat germ: studies with isotopically labeled 3-phospho-D-glycerates showing that the catalyzed reaction is intramolecular. Appendix: phosphoglycerate mutase from wheat germ: isolation crystallization, and properties. Biochemistry 16, 3050–3053.

Lee, Y.H., Ogata, C., Pflugrath, J.W., Levitt, D.G., Sarma, R., Banaszak, L.J., Pilkis, S.J., 1996. Crystal structure of the rat liver fructose-2,6-bisphosphatase based on selenomethionine multiwavelength anomalous dispersion phases. Biochemistry 35, 6010–6019.

Leyva-Vazquez, M.-A., Setlow, P., 1994. Cloning and nucleotide sequence of the genes encoding triosephosphate isomerase, phosphoglycerate mutase and enolase from *Bacillus subtilis*. J. Bacteriol. 176, 3903–3910.

Lively, M.O., El-Maghrabi, M.R., Pilkis, J., D'Angelo, G., Colosia, A.D., Ciavola, J.A., Fraser, B.A., Pilkis, S.J., 1988. Complete amino acid sequence of rat liver 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase. J. Biol. Chem. 263, 839–849.

Magil, N.G., Cowan, A.E., Koppel, D.E., Setlow, P., 1994. The internal pH of the forespore compartment of *Bacillus megaterium* decreases by about 1 pH unit during sporulation. J. Bacteriol. 178, 2252–2258.

Magil, N.G., Cowan, A.E., Leyva-Vazquez, M.-A., Brown, M., Koppel, D.E., Setlow, P., 1996. Analysis of the relationship between the decrease in pH and accumulation of 3-phosphoglyceric acid in developing forespore of *Bacillus species*. J. Bacteriol. 178, 2204–2210.

Meyerhof, O., Kiessling, W., 1935. Biochem. Z. 276, 239–253.

Nairn, J., Krell, T., Coggins, J.R., Pitt, A.R., Fothergill-Gilmore, L.A., Walter, R., Price, N.C., 1995. The use of mass spectrometry to examine the formation and hydrolysis of the phosphorylated form of phosphoglycerate mutase. FEBS Lett. 359, 192–194.

Nairn, J., Price, N.C., Kelly, S.M., Rigden, D., Fothergill-Gilmore, L.A., Krell, T., 1996. Phosphoglycerate mutase from *Schizosaccharomyces pombe*: development of an expression system and characterisation of three histidine mutants of the enzyme. Biochim. Biophys. Acta 296, 69–75.

Ostanin, K., Harms, E.H., Stevis, P.E., Kuciel, R., Zhou, M.M., van Etten, R.L., 1992. Overexpression, site-directed mutagenesis, and mechanism of *Escherichia coli* acid phosphatase. J. Biol. Chem. 267, 22830–22836.

Petitclerc, C., Lazdunski, C., Chappelet, D., Moulin, A., Lazdunski, M., 1970. The functional properties of the Zn2(plus)-and Co2(plus)-alkaline phosphatases of *Escherichia coli*. Labelling of the active site with pyrophosphate, complex formation with arsenate, and reinvestigation of the role of the zinc atoms. Eur. J. Biochem. 14, 301–308.

Poorman, R.A., Randolph, A., Kemp, R.G., Heinrikson, R.L., 1984. Evolution of phosphofructokinase — gene duplication and creation of new effector sites. Nature 309, 467–469.

Price, N.C., Duncan, D., McAlister, J.W., 1985. Inactivation of rabbit muscle phosphoglycerate mutase by limited proteolysis with thermolysin. Biochem. J. 229, 167–171.

Rapport, S., Luebring, J., 1950. J. Biol. Chem. 183, 507–516.

Richardson, J.S., 1981. The anatomy and taxonomy of protein structure. Adv. Protein Chem. 34, 167–339.

Ridgen, D.J., Walter, R.A, Phillips, S.E.V., Fothergill-Gilmore, L.A., 1999. Sulphate ions observed in the 2.12 Å structure of a new crystal form of *S. cerevisiae* phosphoglycerate mutase provide insights into understanding the catalytic mechanism. J. Mol. Biol. 286, 1507–1517.

Ridgen, D.J., Alexeev, D., Phillips, S.E.V., Fothergill-Gilmore, L.A., 1998. The 2.3 Å X-ray crystal structure of *S. cerevisiae* phosphoglycerate mutase. J. Mol. Biol. 276, 449–459.

Ried, T.W., Wilson, I.W., 1976. *E. coli* alkaline phosphatase. In: Boyer, P.D (Ed.), Enzymes, 4. Academic Press, New York, pp. 373–416.

Rose, Z.B., 1971. The phosphorylation of yeast phosphoglycerate mutase. Arch. Biochem. Biophys. 146, 359–360.

Rose, Z.B., 1980. The enzymology of 2,3-bisphosphoglycerate. Adv. Enzymol. Relat. Areas Mol. Biol. 51, 211–253.

Rossmann, M.G., 1981. Evolution of glycolytic enzymes. Phil. Trans. R. Soc. Lond. B 293, 191–203.

Sakata, J., Abe, Y., Uyeda, K., 1991. Molecular cloning of the DNA and expression and characterization of rat testes fructose-6-phosphate,2-kinase:fructose-2,6-bisphosphatase. J. Biol. Chem. 266, 15764–15770.

Sasaki, R., Sugimoto, R., Chiba, H., 1966. Yeast phosphoglyceric acid mutase-modifying enzyme. Arch. Biochem. Biophys. 115, 53–61.

Schneider, G., Lindqvist, Y., Vihko, P., 1993. Three-dimensional structure of rat acid phosphatase. EMBO J. 12, 2609–2615.

Setlow, P., Kornberg, A., 1970. Biochemical studies of bacterial sporulation and germination. XXIII. Energy metabolism in early stages of germination of Bacillus megaterium spores. J. Biol. Chem. 245, 3637–3644.

Singh, R.P., Setlow, P., 1978. Phosphoglycerate mutase in developing forespores of Bacillus megaterium may be regulated by the intrasporal level of free manganous ion. Biochem. Biophys. Res. Commun. 82, 1–5.

Singh, R.P., Setlow, P., 1979. Purification and properties of phosphoglycerate phosphomutase from spores and cells of *Bacillus megaterium*. J. Bacteriol. 137, 1024–1027.

Smith, D.R., Doucette-Stamm, L.A., Deloughery, C., Lee, H., Dubois, J., Aldredge, T., Bashirzadeh, R., Blakely, D., Cook, R., Gilbert, K., Harrison, D., Hoang, L., Keagle, P., Lumm, W., Pothier, B., Qiu, D., Spadafora, R., Vicaire, R., Wang, Y., Wierzbowski, J., Gibson, R., Jiwani, N., Caruso, A., Bush, D., Reeve, J.N., et al., 1997. Complete genome sequence of *Methanobacterium thermoautotrophicum* deltaH: functional analysis and comparative genomics. J. Bacteriol. 179, 7135–7155.

Smith, G.C., Mc. Williams, A.D., Hans, L.F., 1988. Wheat germ phosphoglycerate mutase. Evidence for a metalloenzyme. Biochem. Biophys. Res. Commun. 136, 336–340.

Sowadski, J.M., Handschumacher, M.D., Murthy, M.H.K., Foster, B.A., Wyckoff, H.W., 1985. Refined structure of alkaline phosphatase from *Escherichia coli* at 2.8 Å resolution. J. Mol. Biol. 186, 417–433.

Sternberg, M.J.E., Cohen, F.E., Taylor, W.R., Feldmann, R.J., 1981. Analysis and prediction of structural motifs in the glycolytic enzymes. Phil. Trans. R. Soc. Lond. B 293, 177–189.

Swerdlow, B.M., Setlow, B., Setlow, P., 1981. Levels of H^+ and other monovalent cations in dormant and germinated spores of *Bacillus megaterium*. J. Bacteriol. 148, 20–29.

Tauler, A., Lin, K., Pilkis, S.J., 1990. Hepatic 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase. Use of site-directed mutagenesis to evaluate the roles of His-258 and His-392 in catalysis. J. Biol. Chem. 265, 15617–15622.

Tauler, A., el-Maghrabi, M.R., Pilkis, S.J., 1987. Functional homology of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase, phosphoglycerate mutase, and 2,3-bisphosphoglycerate mutase. J. Biol. Chem. 262, 16808–16815.

Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.

Tsuchiya, Y., Uyeda, K., 1994. Bovine heart fructose 6-P,2-kinase:fructose 2,6-bisphosphatase mRNA and gene structure. Arch. Biochem. Biophys. 310, 467–474.

Ventura, F., Rosa, J.L., Ambrosio, S., Pilkis, S.J., Bartrons, R., 1992. Bovine brain 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase. Evidence for a neural-specific isozyme. J. Biol. Chem. 267, 17939–17943.

Vincent, J.B., Crowder, M.W., Averill, B.A., 1992. Hydrolysis of phosphate monoesters: a biological problem with multiple chemical solutions. Trends Biochem. Sci. 17, 105–110.

Watabe, K., Freese, E., 1979. Purification and properties of the manganese-dependent phosphoglycerate mutase of *Bacillus subtilis*. J. Bacteriol. 137, 773–778.

White, M.F., Fothergill-Gilmore, L.A., 1992. Development of a mutagenesis, expression and purification system for yeast phosphoglycerate mutase. Investigation of the role of active-site His181. Eur. J. Biochem. 207, 709–714.

White, M.F., Fothergill-Gilmore, L.A., Kelly, S.M., Price, N.C., 1993a. Substitution of His-181 by alanine in yeast phosphoglycerate mutase leads to cofactor-induced dissociation of the tetrameric structure. Biochem. J. 291, 479–483.

White, M.F., Fothergill-Gilmore, L.A., Kelly, S.M., Price, N.C., 1993b. Dissociation of the tetrameric phosphoglycerate mutase from yeast by a mutation in the subunit contact region. Biochem. J. 295, 743–748.

Winn, S.I., Watson, H.C., Harkins, R.N., Fothergill, L.A., 1981. Structure and activity of phosphoglycerate mutase. Philos. Trans. R. Soc. Lond. Ser. B 293, 121–130.