Phosphoglycerate mutases

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acids apart in the primary sequence have been switched so as to maintain the same protein fold. Although neither of these residues has been altered to test our understanding of the enzyme's structure, stability elements of prokaryote PGK secondary structure have been substituted for those normally found in yeast. For example, the residues forming this 'nose' region have been deleted from the yeast enzyme by site-directed mutagenesis resulting in an active eukaryotic PGK carrying the most obvious prokaryote PGK structural feature.

The knowledge implicit in a large amino acid sequence database is also of considerable help in making altered protein to study the activity (and presumably) control of the enzyme's function. So far we have altered most of the invariant residues thought to be important for enzymic activity. None of the mutants we have made has had the dramatic enzymic effects expected (see for example [32]). Taken at face value these results would appear to question our knowledge of the PGK structure, although we are fairly sure that had our mutants been of a structural rather than an enzymic kind we would have lost activity. Such an explanation would also avoid the question that can be asked as to why nature has seen fit to conserve such active-site residues throughout evolution if they were not in some way related to activity and therefore subjected to evolutionary pressures. Probably these results question our knowledge concerning enzyme mechanism as it relates to the enzyme's ability to undergo surface-related mutational events without losing activity. In other words, activity can be retained, albeit at much lower levels, without an apparently vital stabilizing residue so long as the overall structure of the enzyme is maintained.

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Phosphoglycerate mutases

LINDA A. FOTHERGILL-GILMORE* and HERMAN C. WATSON†

**Department of Biochemistry, University of Edinburgh, George Square, Edinburgh EH8 9XD, Scotland, U.K. and †Department of Biochemistry, School of Medical Sciences, University of Bristol, Bristol BS8 1TD, U.K.*

Phosphoglycerate mutases comprise a family of enzymes which catalyse reactions involving the transfer of phospho groups among the three carbon atoms of phosphoglycerates. These reactions are essential components in the metabolism of glucose and/or 2,3-bisphosphoglycerate. There are at least four types of phosphoglycerate mutase which are kinetically distinct, but which nevertheless have many features in common (Table 1). These enzymes can be grouped into two distinct sets on the basis of the requirement for the cofactor 2,3-bisphosphoglycerate. The two sets are not homologous to each other, and each set is characterized by the occurrence of isoenzymes. Many aspects of the properties of the phosphoglycerate mutases have recently been reviewed [1].

The cofactor-dependent set of phosphoglycerate mutases include two main types of enzyme: monophosphoglycerate mutase (EC 5.4.2.1) and bisphosphoglycerate mutase (EC

Table 1. *Properties of phosphoglycerate mutases*

Property	Cofactor-dependent		Cofactor-independent	
	Rabbit muscle	Human erythrocyte	Castor plant cytosol	*Bacillus megaterium*
Main reaction	3PGA↔2PGA	1,3BPG→2,3BPG	3PGA↔2PGA	3PGA↔2PGA
Cofactor required	+	+	−	−
M_r	2×30 000	2×30 000	1×64 000	1×61 000
Mn^{2+} required	−	−	−	+
Vanadate inhibition	+	+/−	−	Not known

5.4.2.4/EC 3.1.3.13). These two enzymes are clearly homologous (they share approximately 50% sequence identity) [2–5], but are readily distinguished by the fact that the main reaction each catalyses is different. Thus monophosphoglycerate mutase primarily catalyses the interconversion of 3- and 2-phosphoglycerates, whereas bisphosphoglycerate mutase is mainly active in catalysing the synthesis of 2,3-bisphosphoglycerate from 1,3-bisphosphoglycerate (reviewed in [6]). In fact, both enzymes are rather versatile, and can catalyse the same three reactions, albeit at substantially different relative rates [6].

The cofactor-independent set of phosphoglycerate mutases is also characterized by the presence of two main types. In this case, the principal distinguishing factor is the requirement for Mn^{2+}, as exemplified by the enzyme from *Bacillus* species [7, 8]. Both types of enzyme catalyse only one reaction: the interconversion of 3-phosphoglycerate and 2-phosphoglycerate. Higher plants and filamentous fungi are typical organisms having the cofactor-independent form of phosphoglycerate mutase, and in some higher plants the mutase occurs as at least two isoenzymes corresponding to cytosolic and plastid forms [9–12]. The two isoenzymes have been partially purified from castor oil seeds [12] and have been shown to be of very similar size, but to differ in catalytic properties. The cytosolic enzyme has a greater affinity for both 2-phosphoglycerate and 3-phosphoglycerate, has a lower pH optimum, and has greater heat stability.

The cofactor-independent phosphoglycerate mutases are generally less well characterized than the cofactor-dependent enzymes, and in particular, much less is known of their structures. The remainder of this paper will thus concentrate on the cofactor-dependent group because the availability of detailed structural information means that it is possible to approach an understanding of the evolution and catalytic properties of these enzymes.

The best characterized phosphoglycerate mutase is the enzyme from *Saccharomyces cerevisiae*, and this will be described to provide a structural context for the homologous mammalian isoenzymes. The amino acid sequence of the yeast enzyme is known [13, 14], and the high-resolution three-dimensional structure has been determined [15, 16]. The yeast mutase is a tetramer of identical subunits arranged with almost exact 222 symmetry. The polypeptide backbone folds into a single domain with a core of β-strands surrounded by α-helices (Fig . 1) in a general arrangement typical of all the glycolytic enzymes. The active site was located by using crystals soaked in 3-phosphoglycerate [17] and was found to lie at the bottom of a deep hollow formed entirely by the residues of one subunit. In the tetramer, the four active sites are well separated and appear to be freely accessible to the solvent. In the crystal structure of the unligated native enzyme, two sulphate ions bind in the active site in positions assumed to be occupied by the phospho groups of the ligands.

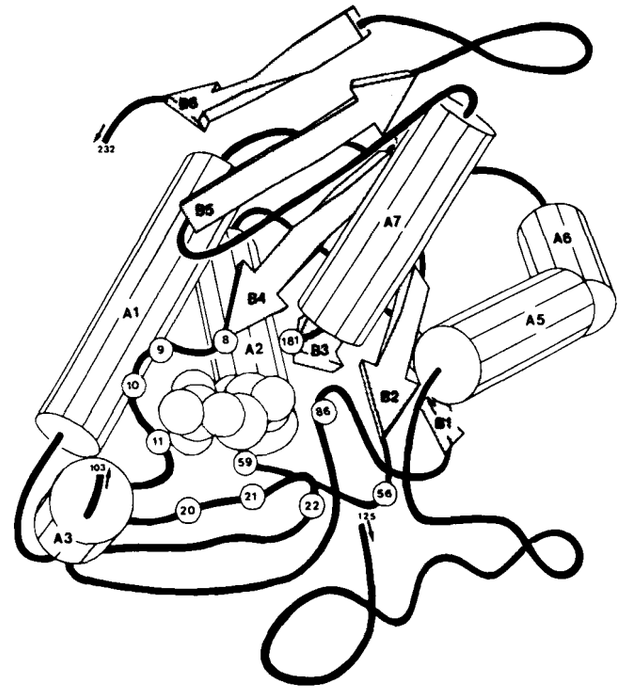

Fig. 1. *The yeast phosphoglycerate mutase subunit*

The elements of regular secondary structure are labelled A1–A3 and A5–A7 for the α-helices (drawn as cylinders), and B1–B6 for the β-strands (drawn as large arrows). The general conformation of the polypeptide backbone connecting the elements of secondary structure is indicated in dark string-like shapes. A portion of the polypeptide chain including helix 4 has been removed to reveal the α-carbon positions of residues at the active site, as well as the position occupied by bound ligands as indicated by the space-filling drawing of 3-phosphoglycerate located by crystal-soaking experiments. The *C*-terminal tail extends 14 residues beyond the crystallographic *C*-terminus shown in the upper left of the drawing.

The most prominent features of the active site are two histidine side-chains, which are parallel and about 0.4 nm apart. His-8 is at the carboxyl end of strand 4 of the β-sheet, and His-181 is at the carboxyl end of strand 3 (Fig. 1). An arginine (residue 7) forms part of this side-chain complex, with one of its guanidinium nitrogens near the 5-nitrogens of the two histidines. One sulphate binds close to His-8 and is located in a position where it can also make hydrogen bonds with Ser-11 and Thr-20. The second active-site sulphate interacts with the positive charge associated with the

dipole at the *N*-terminus of helix 7. Arg-59 is buried deep in the active-site pocket, where it forms a salt-bridge with the carboxyl group of the bound substrates.

The *C*-terminal 14 residues of yeast phosphoglycerate mutase are not observed in the electron density map, presumably because they constitute a flexible tail in the non-phosphorylated, non-ligated form of the enzyme that was crystallized. These residues are of considerable importance, because they are required for activity. The *C*-terminal tail is particularly susceptible to proteolysis, and a phosphoglycerate mutase molecule which has lost these residues, but is otherwise apparently unmodified, shows complete loss of mutase activity. These observations have been noted for both the yeast enzyme [15, 18] and the rabbit muscle enzyme [19].

The observation that phosphoglycerate mutase isolated from adult mammalian muscle is much more sensitive to inhibition by Hg^{2+} than is the enzyme from other mammalian tissues, suggested that phosphoglycerate mutase occurs as tissue-specific isoenzymes [20]. Moreover, the sensitivity of the muscle isoenzyme increases during embryological and postnatal development, showing that the expression of the isoenzymes in muscle is developmentally regulated [21]. The existence of isoenzyme forms of phosphoglycerate mutase has been shown for all vertebrates examined (mammals, birds, reptiles, amphibians and fish) [22, 23], whereas the distribution of the erythrocyte form (bisphosphoglycerate mutase) is narrower. The latter enzyme appears to be absent from fish and occurs in very low amounts in certain mammals such as some carnivores (e.g. civets and lions) and artiodactyls (e.g. cattle and goats) (reviewed in [1]).

Three genes encoding phosphoglycerate mutase isoenzymes exist in mammals, which correspond to the muscle, brain and erythrocyte forms [24–26]. In tissues where more than one gene is active, multiple isoenzymes occur which correspond to homo-and hetero-dimers [27]. The muscle and brain forms have very similar kinetic properties [28], but differ in their susceptibility to inactivation by Hg^{2+}, heat or thiol reagents [20, 29, 30]. The erythrocyte form is readily distinguished from the other forms by the relative ease with which it catalyses the synthesis of 2,3-bisphosphoglycerate. Thus the catalytic constant for this reaction is 12.5 s^{-1} for the erythrocyte enzyme and 0.4 s^{-1} for the muscle form [6]. In contrast, the muscle enzyme has a higher catalytic constant for the glycolytic/gluconeogenic reaction (1330 s^{-1}) than does the erythrocyte enzyme (1.7 s^{-1}) [6].

Complete amino acid sequences are available for all three human isoenzymes [2–5], for rabbit erythrocyte bisphosphoglycerate mutase [31], as well as for the yeast enzyme [13, 14]. The sequences are all clearly homologous, and can be readily aligned with only two small gaps (see [1]). Overall sequence comparisons are given in Table 2. In general, glycolytic enzymes are evolving at the rate of about 5% of the residues changing per 100 million years [32]. Phosphoglycerate mutase conforms to this pattern as is indicated in Table 2. Sequence comparisons among the three human isoenzymes show that the muscle and brain isoenzymes are much more similar to each other than to the erythrocyte enzyme. This is good evidence that the gene duplication event giving rise to the erythrocyte form pre-dates the divergence of the muscle and brain isoenzymes. It is known that amphibia use bisphosphoglycerate as a regulator of haemoglobin oxygen affinity, but fish do not (reviewed in [33]). It is, therefore, probable that the divergence of the monophospho- and bisphosphoglycerate mutases occurred between 400 million years ago (fish–mammal divergence) and 300 million years ago (amphibia–mammal divergence). Because monophosphoglycerate mutase and bisphosphoglycerate mutase have about the same rate of evolution, it is reasonable to suggest that the divergence of monophosphoglycerate mutase into the muscle and brain isoenzymes occurred after the bird–mammal divergence (300 million years ago) and before the radiation of the mammalian orders (100 million years ago).

Table 2. *Overall sequence comparisons*

The number of residues compared relates only to matched sequences; deletions are not included. Abbreviations: Y, yeast monophosphoglycerate mutase; HM, human muscle monophosphoglycerate mutase; HB, human brain monophosphoglycerate mutase; HE, human erythrocyte bisphosphoglycerate mutase; RE, rabbit erythrocyte bisphosphoglycerate mutase.

Sequences compared	No. of residues compared	No. of differences	Differences (%)
Y-HM	246	119	48
Y-HB	246	121	49
Y-HE	246	126	51
HE-RE	258	6	2.3
HM-HB	252	53	21
HM-HE	252	123	49
HB-HE	253	118	47

The availability of the amino acid sequences of the isoenzymes, together with the strongly homologous yeast tertiary structure, enables many of the properties of the isoenzymes to be interpreted in structural terms. Thus the susceptibility of the muscle isoenzyme to thiol reagents is probably due to a cysteine at the active site (residue 20), which is a serine in the brain isoenzyme. The structural basis for the different kinetic properties of the erythrocyte isoenzyme is of considerable interest. The amino acid sequence shows that most of the active-site residues are conserved (e.g. His-8, His-181, Arg-7 and Arg-59). However, notable differences occur at residue 11, which is in a suitable position in the yeast enzyme to provide a ligand for the phospho group at the active site. In the monophosphoglycerate mutases, this residue is either a serine or a threonine, but is replaced by a glycine in bisphosphoglycerate mutase. The fact that the erythrocyte isoenzyme thus has one fewer phospho ligand is consistent with the greater instability of its phosphorylated form [34] and is also consistent with the relative ease with which the product 2,3-bisphosphoglycerate can dissociate [6]. It is likely that sequence differences at the *C*-terminal tail may also contribute to variations in ligand binding. The differences in catalytic properties between the different isoenzymes of phosphoglycerate mutase are currently being probed by site-directed mutagenesis [35].

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Isoenzymes of pyruvate kinase

HILARY MUIRHEAD
Department of Biochemistry, University of Bristol, Bristol BS8 1TD, U.K.

Introduction

Pyruvate kinase (PK, EC 2.7.1.40) is of particular importance in glycolysis for controlling the flux from fructose 1,6-bisphosphate (FBP) through to pyruvate. It catalyses the conversion of phosphoenolpyruvate into pyruvate by the addition of a proton and the loss of a phospho group that is transferred to ADP and requires both bivalent and monovalent cations:

$$\text{Phosphoenolpyruvate} + \text{MgADP} + \text{H}^+ \xrightarrow{\text{Mg}^{2+},\text{K}^+} \text{Pyruvate} + \text{MgATP}$$

The enzyme has a low nucleotide specificity and the 5′-diphosphates of guanosine, inosine, uridine and cytidine can all serve as phospho acceptors. The properties of the enzyme have been reviewed by Muirhead [1].

This paper discusses work which has been carried out in several different laboratories. PK has been isolated from a variety of cells and tissues and in most cases, whether derived from bacterial, plant or animal sources, the enzymes are tetramers of identical subunits, each with at least 500 amino acid residues. In vertebrate tissues four different isoenzymes are found. These isoenzymes are tissue specific, have kinetic properties reflecting the different metabolic requirements of the tissues and differ in the regulation of gene expression [2]. The M1 type is the major form of adult skeletal muscle, heart and brain and shows predominantly hyperbolic Michaelis–Menten kinetics. The M2 type is the only form detected in early fetal tissues and is present in most adult tissues. The R type is expressed only in erythrocytes and the L type is the major form in adult liver. In contrast to M1 the M2, R and L types are all allosterically regulated and show sigmoidal kinetics with respect to the substrate phosphoenolpyruvate. In addition, R and L are regulated by reversible protein kinase-mediated phosphorylation. These kinetic properties can be explained by the existence of several different conformational states for the tetrameric enzyme ranging from a T-state with low affinity to an R-state with high affinity for substrate [3]. The high-affinity R-state is stabilized by phosphoenolpyruvate, FBP and low pH. The low-affinity T-conformation is stabilized by ATP, alkaline pH, gluconeogenic amino acids such as alanine and the phosphorylation of a specific serine (present in R and L) near the *N*-terminus.

The crystal structure of an M1 isoenzyme is known [4] and amino acid sequences are available for M1, M2, L and R isoenzymes [4–9]. Residue numbers for M1 are used throughout this paper. Alignment of these sequences shows them to be strongly conserved and implies that the tertiary and quaternary structures of all the isoenzymes are very similar. Sequence variability in the inter-subunit contact areas may affect the equilibrium between the R and T quaternary structures and give rise to the different kinetic properties.

Abbreviation used: PK, pyruvate kinase.

Gene structure and expression

Genetic studies have suggested the presence of two structural genes in mammals: one coding for M1 and M2 and one coding for L and R [10, 11]. The organization of the gene has been determined for chicken M1 [12], rat L and R [8, 13] and rat M1 and M2 [5]. In general, there is a high degree of homology between the different genes in both gene organization and exon sequence. With the exceptions discussed below the genes have the same number of exons and corresponding exons are of the same length and similar sequence. However, the introns are of quite different lengths. The introns do not occur at random positions, but tend to be located in regions connecting elements of regular secondary structure of the protein [4, 12]. This suggests that the genes have diverged from a common ancestral gene and that the coding region evolved very slowly. In contrast, the absence of