**Allomorphy as a mechanism of post-translational control of enzyme activity**

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Enzyme regulation is vital for metabolic adaptability in living systems. Fine control of enzyme activity is often delivered through post-translational mechanisms, such as allosterism or allomorphy. β-phosphoglucomutase (βPGM) from *Lactococcus lactis* is a phosphoryl transfer enzyme required for complete catabolism of trehalose and maltose, through the isomerisation of β-glucose 1-phosphate to glucose 6-phosphate via β-glucose 1,6-bisphosphate. Surprisingly for a gatekeeper of glycolysis, no fine control mechanism of βPGM has yet been reported. Herein, we describe allomorphy, a post-translational control mechanism of enzyme activity. In βPGM, isomerisation of the K145-P146 peptide bond results in the population of two conformers that have different activities owing to repositioning of the K145 sidechain. In vivo phosphorylating agents, such as fructose 1,6-bisphosphate, generate phosphorylated forms of both conformers, leading to a lag phase in activity until the more active phosphorylated conformer dominates. In contrast, the reaction intermediate β-glucose 1,6-bisphosphate, whose concentration depends on the β-glucose 1-phosphate concentration, couples the conformational switch and the phosphorylation step, resulting in the rapid generation of the more active phosphorylated conformer. In enabling different behaviours for different allomorphic activators, allomorphy allows an organism to maximise its responsiveness to environmental changes while minimising the diversion of valuable metabolites.

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Enzyme regulation is vital in maintaining the balance of catabolism and anabolism in living systems. Enzyme activity is subject to precise control, sometimes involving manifold layers of regulation, and failure often results in metabolic disorders and disease. Regulatory mechanisms are divided into two broad categories: those relating to the control of enzyme concentration (coarse control) and those that modulate enzyme activity (fine control). In coarse control, concentration is determined by transcriptional modulation of gene expression and the balance between the rates of translation and degradation, with additional contributions from maturation, cellular compartmentalisation and local co-clustering. Coarse control occurs on relatively long timescales (hours to days). In fine control, a diverse group of regulatory mechanisms act to modulate enzyme activity over much shorter timescales (<second to minutes). This group includes the binding of regulatory molecules and reversible covalent modification, and often involves allosteric modulation, where an effector, acting somewhere other than the active site, stabilises forms of the enzyme with a reduced or enhanced activity. Alternatively, allokyria is a fine control mechanism, where the activity of a monomeric enzyme is modulated by the near-equivalence of the conformational exchange rate and the catalytic rate in a substrate concentration-dependent manner.

Precise enzyme regulation allows organisms to be responsive to environmental changes and to exploit multiple energy sources. Lactococcus lactis (L. lactis) is a Gram-positive bacterium that has worldwide usage in the manufacture of fermented dairy products and in the commercial production of lactic acid. It can grow on a variety of carbohydrate media including trehalose and maltose.

Trehalose is transported into L. lactis by the phosphoenolpyruvate-dependent phosphotransferase system, yielding trehalose 6-phosphate (T6P), which is phosphorolysed by β-glucose 1-phosphate phosphatase (βG1P) and glucose 6-phosphate (G6P). Glucose is subsequently phosphorylated to G6P by glucokinase and enters glycolysis via fructose 1,6-bisphosphate (F16BP). For complete catabolism of both trehalose and glucose, the isomerisation of β-G16BP in the distal site. The phosphoryl transfer reaction between βG1P and G1P allows PGM to reach its catalytic rate in a substrate concentration-dependent manner. Correspondingly, both Pγ-dependent trehalose 6-phosphate phosphorylase and Pδ-dependent maltose phosphorylase (Supplementary Fig. 1) operate in the reverse sense to their physiological roles in wild-type L. lactis, resulting in βG1P being combined with G6P to form T6P or polymerised to form amylose (α(1→4)-linked glucose units). In trehalose and maltose metabolism, therefore, PGM acts as the gatekeeper to and from glycolysis, and is expected to be subject to tight regulation. In terms of coarse control, transcription of the PGM gene (pgmB), which is located in the tre operon, is subject to negative transcriptional control by glucose and lactose. When L. lactis switches from metabolising glucose to metabolising maltose (or by implication, trehalose), there is a significant rise in the specific activity of PGM over a period of several hours. However, no fine control mechanism has yet been identified at basal levels of PGM, which would allow the cell to compete more successfully during a transition between carbohydrate sources.

PGM is a monomeric magnesium-dependent phosphoryl transfer enzyme of the haloacid dehalogenase (HAD) superfamily. The active site is located in the cleft between the α/β core domain (M1–D15, S88–K216) and the α-helical cap domain (T16–V87), with closure of the cleft through domain reorientation occurring during catalysis. Two phosphate group binding sites are present, a proximal site adjacent to the carbohydrate nucleophile and the catalytic Mg2+ ion, and a distal site located ~8 Å away in the closed enzyme. During steady-state catalysis, βG1P binds to phosphorylated PGM (PGMP, phosphorylated on D8) and forms β-glucose 1,6-bisphosphate (βG16BP). Release to solution and subsequent rebinding of βG16BP in the alternate orientation leads to the formation of G6P and the regeneration of PGM. In vitro, a phosphorylating (priming) agent is required to initiate the catalytic cycle since the half-life of PGMP is ~30 s. In vivo, potential candidates for this agent include F16BP, βG1P, G6P, α-glucose 1,6-bisphosphate (αG16BP) and acetyl phosphate (AcP), as well as the reaction intermediate, βG16BP. However, only βG16BP allows PGM to reach its maximum catalytic rate, and a significant lag phase is observed in the reaction with αG16BP as the phosphorylating agent, until the βG16BP concentration greatly exceeds its resting concentration in the cell. In the current kinetic model for PGM catalysis, αG16BP is also required to act as a very strong inhibitor of PGM. αG16BP is a close structural analogue of βG16BP, but very similar kinetic behaviour is observed when AcP is used as the phosphorylating agent, suggesting that other factors are

**Fig. 1** βPGM catalytic cycle. βPGM reaction scheme for the enzymatic conversion of βG1P to G6P via a βG16BP intermediate. The phosphoryl transfer reaction between phospho-enzyme (βPGMP, phosphorylated at residue D8) and βG1P is illustrated with the transferring phosphate (blue) in the proximal site and the 1-phosphate (red) of βG1P in the distal site. The phosphoryl transfer reaction between βPGM and βG16BP is shown with the transferring phosphate (red) in the proximal site and the 6-phosphate (blue) of βG16BP in the distal site.
analogue of AP, and the Mg$_2$βδA113.

A conformer B (BP) and a exchange.

termed allomorphy to illustrate its relationship to and distinction

lography, site-directed mutagenesis and kinetic assays, that a

through combined use of NMR spectroscopy, X-ray crystal-

tributing to post-translational control of

Results

βPGM$_{WT}$ exchanges slowly between two stable conformations.

The observation of a lag phase when using either αG16BP or AcP$_{28,31}$ as the phosphorylating agent implies that the target of phosphorylation, the substrate-free enzyme, has a role in post-translational control. Hence, the solution properties of substrate-free wild-type βPGM (βPGM$_{WT}$) were investigated using NMR spectroscopy. In the previous backbone resonance assignment of βPGM$_{WT}$ (BMRB 7235$^{26}$) performed in standard NMR buffer (50 mM K$^+$ HEPES (pH 7.2), 5 mM MgCl$_2$, 2 mM Na$_2$H$_4$ 10% (v/v) $^2$H$_2$O and 1 mM trimethylsilyl propanoic acid (TSP)) containing 10 mM NH$_4$F, two features were apparent during the analysis: (1) peaks of 30 active site residues were missing from the spectra owing to line-broadening resulting from conformational exchange on the millisecond timescale and (2) a large number of unassigned $^{13}$C resonances were present with a low intensity. To test whether HEPES or NH$_4$F were contributing to the millisecond conformational exchange, spectra were recorded in tris buffer (50 mM tris (pH 7.2), 5 mM MgCl$_2$, 2 mM Na$_2$H$_4$ 10% (v/v) $^2$H$_2$O and 1 mM TSP) and mixtures of HEPES and tris buffers in order to transfer the assignment between conditions$^{32}$. It was noticed that the inclusion of 5 mM tris in the standard NMR buffer increased the intensity of the unassigned resonances significantly and therefore all observable resonances in the spectra were re-assigned using standard triple resonance TROSY-based methodology$^{34}$. Excluding the ten proline residues and the N-terminal methionine, the backbone resonances of 193 out of a total of 210 residues (92%) were assigned. Seventeen residues located in the vicinity of the active site remained unassigned (L9, D10, G11, R38, L44, K45, G46, S48, R49, E50, D51, S52, L53, K117, N118, D170 and S171). Notably, 102 of the assigned residues displayed pairs of resonances in the $^{1}$H$^{15}$N-TROSY spectrum (Fig. 2a, Supplementary Fig. 2a), consistent with the population of two βPGM$_{WT}$ conformers (70% conformer A, BMRB 28095 and 30% conformer B, BMRB 28096). A further five residues (K145, A147, D149, I150 and Q176) have assignments in conformer A, but are missing assignments in conformer B, owing to some differential millisecond conformational exchange occurring in the two species. The βPGM$_{WT}$ conformers are present in the spectra as a result of slow conformational exchange rather than as chemically distinct species, as the addition of 3 mM BeCl$_2$ and 10 mM NH$_4$F to the βPGM$_{WT}$ sample induced the population of a single βPGM$_{WT}$:BeF$_3$ complex (an analogue of phosphorylated conformer A; BMRB 17851$^{35}$) (Supplementary Fig. 3a). The exchange between conformer A and conformer B is on the multi-second timescale, with $k_{ex}$ $<$ 1.0 s$^{-1}$ from ZZ-exchange measurement. Differences in chemical shift between the two conformers (Supplementary Fig. 4a) indicate that the regions of βPGM$_{WT}$ involved in the multi-second conformational exchange process are located primarily in the core domain and comprise the D137–A147 loop, the β-strands (K109–A113 and D133–A136) at the outer edge of the isomerisation of proline 146 results in the near-equal population of two conformers that have different activities. Alternative phosphorylating agents such as F16BP and AcP generate phosphorylated forms of both conformers, resulting in a lag phase in βPGM activity until the more active phosphorylated conformer dominates. In contrast, the βG16BP reaction intermediate is able to couple the conformational switch and the phosphorylation step, resulting in the rapid generation of the more active phosphorylated species. This allows the βG16BP concentration to effectively act as a surrogate of the βGIP concentration and modulate the activity of βPGM according to the carbohydrate source available to L. lactis.

![Fig. 2 Effect of different phosphorylating agents on βPGM. a Overlays of a section of $^{1}$H$^{15}$N-TROSY spectra highlighting the behaviour of residue A113. b βPGM$_{WT}$ (black) populates conformer A and conformer B in slow exchange. βPGM$_{WT}$ supplemented with F16BP (pink) populates phosphorylated conformer A (A$^{\alpha}$) as the dominant species, phosphorylated conformer B (B$^{\beta}$) and a βPGM$_{WT}$:F16BP species (A:F16BP). βPGM$_{WT}$ supplemented with G16BP (green) populates an A:βG16BP complex. βPGM$_{F146A}$ (black) populates one conformer (conformer B). βPGM$_{F146A}$ supplemented with F16BP (pink) populates conformer B and B$^{\beta}$. βPGM$_{F146A}$ supplemented with G16BP (green) populates a A$^{\alpha}$:G6P complex and a B:βG16BP complex. Peaks indicated by grey asterisks correspond to the βPGM$_{WT}$:BeF$_3$ complex (grey; $\delta_N$ = 133.5 ppm; BMRB 17851$^{35}$), which is an analogue of A$^{\alpha}$, and the Mg$^{2+}$-saturated βPGM$_{G16BP}$ complex (grey; $\delta_N$ = 133.8 ppm; BMRB 27174$^{35}$), which is a mimic of the A:βG16BP complex, and are shown for comparison.](image-url)
The population distribution of conformer A and conformer B was exchange (2WHE29) showing residues of βPGMWT undergoing conformational exchange on different timescales. Residues that populate two conformations in slow exchange are coloured in shades of blue according to chemical shift differences between conformer A and conformer B, with the intensity of colour and thickness of the backbone corresponding to larger values. Residues in conformer A and conformer B with missing backbone amide peaks in the \( ^{1}H^{15}N\)-TROSY spectrum of βPGMWT are coloured black, whereas missing backbone amide peaks in conformer B only are coloured purple. The amide \( ^{1}H^{15}N\) coherences are likely broadened beyond detection owing to intermediate exchange on the millisecond timescale. The catalytic Mg\(^{2+}\) ion is indicated as a green sphere.

**β-sheet and the I152–S163 and Q172–A183 α- and 3\( _{10}\)-helical regions (Fig. 3).** Predicted random coil index order parameters (RCI-S\(^{2}\))\(^{36}\) show a decrease in value for conformer B in two regions (G32–R38 in the cap domain and D133–K145 in the core domain) (Supplementary Fig. 5a), which indicates increased conformational flexibility compared with conformer A.

**Influence of physiological factors on the conformational exchange.** An investigation of factors that could potentially affect the population distribution of conformer A and conformer B was performed using \( ^{1}H^{15}N\)-TROSY spectra of βPGMWT recorded under different conditions of temperature, pH, hydrostatic pressure, MgCl\(_{2}\) (0–100 mM), NaCl (0–200 mM), K\(^{2+}\) HEPES buffer (0–200 mM) and βPGMWT concentration (0.1–1.2 mM). All of these perturbations had little or no effect, apart from the addition of either MgCl\(_{2}\) (100 mM) or NaCl (200 mM) to standard NMR buffer, which shifted the population of βPGMWT primarily to conformer A (Supplementary Fig. 6a–d). Buffer exchange into deionised water resulted in conformer B being the dominant population. However, both conformer A and conformer B remained populated when Mg\(^{2+}\) was removed from the NMR buffer solution, showing that the multi-second conformational exchange is not simply a result of incomplete saturation of the catalytic Mg\(^{2+}\)-binding site. These observations indicate that chloride anions perturb the population distribution.

The inorganic ionic composition of \( L.\) lactis cytoplasm (~2 mM Mg\(^{2+}\), ~50 mM Na\(^{+}\), ~400 mM K\(^{+}\), ~50 mM Cl\(^{−}\))\(^{37}\) overlaps with the concentration ranges tested, where the population distribution between conformer A and conformer B remained unaffected. Therefore, it is expected that both conformer A and conformer B are populated in cytoplasm. However, the intracellular milieu is a complex mix of metabolites that could influence this equilibrium. This environment was mimicked through the use of bovine skimmed milk, which had been filtered to remove species with a molecular weight larger than 10 kDa. The \( ^{1}H^{15}N\)-TROSY spectrum revealed that both conformer A and conformer B were populated with a similar ratio (60% conformer A and 40% conformer B) to βPGMWT recorded in standard NMR buffer (Supplementary Figs. 2c, 7a). However, minor chemical shift changes in the active site loops and the sharpening of some peaks that were line-broadened under standard conditions indicated that one of the milk components was binding in the vicinity of the active site. The two dominant organic components of the filtered milk were lactose and citrate (Supplementary Fig. 2d). Titration of lactose into βPGMWT had no effect on the \( ^{1}H^{15}N\)-TROSY spectrum, whereas titration of citrate led to equivalent chemical shift changes and sharpening of line-broadened peaks to those observed in milk. Similar effects were observed in both conformer A and conformer B. Hence, βPGMWT was crystallised in the presence of citrate and the structure was determined to 2.1 Å resolution (PDB 6YDL, Supplementary Fig. 8a–c; Supplementary Table 1). Two chains are present in the crystallographic asymmetric unit, one of which has citrate and acetate bound, whilst the other has tris and acetate bound. Citrate is coordinated in the active site by residues T16, H20, V47 and R49 and A115–K117 and mimics substrate binding to some extent. Both monomers share a similar fold and overlay closely with a previously reported substrate-free βPGMWT structure (PDB 2WHE29; non-H atom RMSDs of 0.56 Å and 0.95 Å). Although only one of the two conformers observed in solution is represented in the crystal, the NMR experiments show that both conformer A and conformer B remain well-populated under physiological conditions.

The conformational exchange involves \textit{cis}-\textit{trans} proline isomerisation. Exchange phenomena on multi-second timescales in proteins are often a consequence of \textit{cis}-\textit{trans} isomerisation of Xaa-Pro peptide bonds\(^{39,40}\). The largest differences in chemical shift between conformer A and conformer B are observed for residues in a loop (D137–A147) containing two proline residues (P138 and P146) (Supplementary Fig. 4a). From the crystal structures of the substrate-free form of the enzyme (PDB 6YDL (Supplementary Table 1) determined to 1.5 Å resolution, which compares closely with PDB 2WHE29 (non-H atom RMSD = 0.53 Å) and PDB 1Z0I\(^{25}\) (non-H atom RMSD = 0.65 Å), nine \textit{trans} Xaa-Pro peptide bonds are present in βPGMWT, whereas the K145–P146 peptide bond adopts a \textit{cis} conformation. Proline residues with \textit{cis} peptide bonds have \( ^{13}C\beta \) nuclei that resonate 2.0–2.5 ppm downfield from those with \textit{trans} peptide bonds\(^{41}\) and therefore the isomerisation state of the Xaa-Pro peptide bonds for βPGMWT in solution was investigated. All but one of the assigned proline residues in conformer A and conformer B possess \( ^{13}C\beta \) chemical shifts in the range 30.4–31.9 ppm consistent with the population of \textit{trans} Xaa-Pro peptide bonds (Supplementary Fig. 5b). In contrast, the \( ^{13}C\beta \) chemical shift for P146 (34.7 ppm) corroborates the presence of a \textit{cis} K145–P146 peptide bond in solution for conformer A. However for conformer B, the absence of proline \( ^{13}C\beta \) resonances for P146 and P148, owing to millisecond conformational exchange in the K145–I150 region, precluded an identification of the isomerisation state for these proline residues using NMR methods. To explore whether proline isomerisation at the K145–P146 peptide bond is the source of the multi-second conformational exchange in βPGMWT, the βPGM variant P146A (βPGM\(_{P146A}\)) was prepared and the solution properties of the substrate-free form were investigated. A \( ^{1}H^{15}N\)-TROSY spectrum shows that only a single species is present (Fig. 2b, Supplementary Fig. 2b).
and 194 out of a total of 211 residues (92%) were assigned using standard TROSY-based methodology (BMRB 27920). The same seventeen residues as βPGMWT remain unassigned owing to millisecond conformational exchange. The chemical shifts of βPGMP146A were compared with those of conformer A and conformer B of βPGMWT (Supplementary Fig. 4b, c). Although the largest perturbations relate to the mutation site (together with G182 and S171 residues), the remaining chemical shifts of the K145 sidechain in βPGMP146A changes the isomerisation state of the K145 sidechain in βPGMWT (as conformer A) and βPGMP146A superposed on the core domain. The catalytic Mg\(^{2+}\) ion is drawn as a green sphere, black dashes indicate metal ion coordination and orange dashes show probable hydrogen bonds.

The D137–A146 peptide bond allows coordination of the K145 sidechain by E169 and A113, whereas in βPGMP146A a trans K146–A146 peptide bond changes significantly the backbone conformation of the D137–A147 loop, which precludes active site engagement of the K145 sidechain. The most pronounced consequence of the change in isomerisation state of the K145 sidechain in βPGMP146A to engage in the active site conformation in the absence of residues S144–P148 in βPGMP146A isomerisation state remains ambiguous using NMR methods. Therefore, the solution conformations for these species are closely similar. However, although the K145–A146 peptide bond in βPGMP146A is likely to adopt a trans conformation as the dominant population, the isomerisation state remains ambiguous using NMR methods. Therefore, ε-amino group of K145 is coordinated by the carbonyl oxygen atom of A113, the carboxylate sidechain of E169, and a water molecule that is replaced in the transition state (Fig. 4a). Instead, this sidechain is positioned in the open conformation of the transition state.

The electron density is best fit by the trans conformation of the K145–A146 peptide bond (ω dihedral angle = −177°) (Fig. 4a, c). In comparison with βPGMWT, the D137–A147 loop adopts a different conformation, although both a 3_10-helix (D137–V141) and a β-turn hydrogen bond (A147_HN–S144_CO3_) are retained. These perturbations in structure are consistent with the chemical shift changes observed between conformer A and βPGMP146A and support the βPGMP146A structure being a close model of conformer B.

The most pronounced consequence of the change in isomerisation state of the K145–A146 peptide bond is the failure of the K145 sidechain in βPGMP146A to engage in the active site conformation in the transition state (Fig. 4a). Instead, this sidechain is positioned in the open conformation in the absence of residues S144–P148 in βPGMP146A. The electron density is best fit by the trans conformation of the K145–A146 peptide bond (ω dihedral angle = −177°) (Fig. 4a, c). In comparison with βPGMWT, the D137–A147 loop adopts a different conformation, although both a 3_10-helix (D137–V141) and a β-turn hydrogen bond (A147_HN–S144_CO3_) are retained. These perturbations in structure are consistent with the chemical shift changes observed between conformer A and βPGMP146A and support the βPGMP146A structure being a close model of conformer B.

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of G6P production was measured indirectly using a glucose 6-phosphate
are included in the kinetic pro-

β

isation of the K145

formers to the isomerisation state of the K145-X146 peptide

The predicted RCI-S² order parameters for

β

mirror those of conformer B in

PGMWT hydrogen bonding are satis-

PGMP, occurring at a rate proportional to the amount of enzyme.

the position of the missing ε-amino group of K145 and its

β

PGMWT hydrogen bonding are satisfied by a water molecule.

The predicted RCI-S² order parameters for βPGM146A

and βPGMWT (Supplementary Fig. 5a, c) share similar profiles apart

from the D137–A147 loop region, where the RCI-S² values for

βPGM146A indicate increased dynamic properties that broadly

mirror those of conformer B in βPGMWT. Together, these data

reveal that βPGM146A reflects the properties of conformer B, and

link the chemical shift and RCI-S² differences between confor-

mers to the isomerisation state of the K145-X146 peptide

bond. Thus, the multi-second exchange between conformer A

and conformer B in solution involves cis–trans proline isomeri-

sation of the K145–P146 peptide bond.

βPGMWT lag phase depends on the phosphorylating agent. To

ensure that the extent of the lag phase observed previously with

Acp is not a method dependent observation31, the effect of dif-

ferent phosphorylating agents on the mutase activity of βPGMWT

was investigated by monitoring the conversion of 50 µM βG1P to

G6P with either F16BP (1 mM), AcP (8 mM) or βG16BP (10 µM)

present as phosphorylating agents, using the standard glucose 6-

phosphate dehydrogenase coupled assay28. Despite the crucial

involvement of βG16BP as the reaction intermediate in the

catalytic cycle, its concentration in the cell can vary markedly and

is dependent upon the concentration of βG1P. Therefore, F16BP

is the most likely phosphorylating agent of βPGMWT in vivo

when L. lactis is growing on glucose-rich media (~50 mM

F16BP [17] versus Kₘ ~100 µM [25]). AcP is also a potential activator

in vivo, as although it is present at lower concentrations (1–3 mM

in Escherichia coli [E. coli] [43, 44] versus Kₘ ~800 µM [25]), it is

inherently a much faster phosphorylating agent. In the coupled

assay experiments with βPGMWT (Fig. 5a), when either F16BP or

AcP was used as the phosphorylating agent, their progression

curves display significant lag phases. The lag is considerably more

pronounced in the F16BP experiment, and consequently the

maximum rate of βG1P to G6P conversion is not achieved before

the substrate is exhausted. When βG16BP was used as the

phosphorylating agent the kinetic profile shows a linear, fast

initial rate. Consequently, initial rate measurements were made

at several βG1P and βG16BP concentrations (10–700 µM and

0.4–100 µM, respectively) and were globally fitted to an equation

derived for a ping-pong mechanism with βG1P inhibition28.

Accurate fits were obtained at βG16BP concentrations up to 10

µM, as above this concentration the model no longer describes

the data. At elevated βG16BP concentrations, the back reaction

from βG16BP to βG1P becomes significant, and the free βG16BP

concentration is attenuated owing to a multimeric interaction

between βG16BP and Mg²⁺ ions31. Accordingly, the data above

exceeding 10 µM βG16BP were omitted from the fitting. This analysis

yielded values for kₗag of 382 ± 12 s⁻¹, Kₘ (βG1P) of 91 ± 4 µM,

Kₘ (βG16BP) of 8.5 ± 0.3 µM and Kᵢ (βG1P) of 1510 ± 100 µM

(Supplementary Fig. 9a). These values are all higher than those

previously reported25,28 for βPGMWT owing to the extension of

the analysis to higher βG1P and Mg²⁺ concentrations.

βPGM catalysis utilises a cis K145-X146 peptide bond. To

assess whether βPGM is active as conformer B, the effect of dif-

ferent phosphorylating agents on the mutase activity of

βPGM146A was investigated by monitoring the conversion of

50 µM βG1P to G6P with either F16BP (1 mM), AcP (8 mM) or

βG16BP (10 µM) present as phosphorylating agents using the

standard glucose 6-phosphate dehydrogenase coupled assay. As

for βPGMWT, the kinetic profiles for βPGM146A display sig-

nificant lag phases with F16BP and AcP, whereas the progression

curve with βG16BP shows a linear, fast initial rate (Fig. 5b). Conse-

quently, initial rate measurements were made at several

βG1P and βG16BP concentrations (5–500 µM and 2–100 µM,

respectively) and were globally fitted to the equation used for

βPGMWT above. For βPGM146A, the fitting yielded values for kₗag

of 19.2 ± 0.2 s⁻¹, Kₘ (βG1P) of 157 ± 3 µM, Kₘ (βG16BP) of

175 ± 3 µM and Kᵢ (βG1P) of 933 ± 32 µM (Supplementary Fig. 9b).

In addition, the equilibration of βG1P and G6P for both βPGMWT

and βPGM146A was monitored by 3¹P NMR spectroscopy using

AcP as a phosphorylating agent (Supplementary Fig. 9c, d)31. The

time courses show a similar overall profile together with the

presence of the lag phase and subsequent fitting of the linear

segments yielded a kₗobs of 70 ± 3 s⁻¹ for βPGMWT and a kₗobs of

1.1 ± 0.2 s⁻¹ for βPGM146A. The variation between the kinetic

parameters derived using the two methods is caused by inhibition

resulting from different levels of phosphate-containing species

present in the assays. However, the data clearly demonstrate that

βPGM146A is active, with a ~20-fold reduction in kₗcat a ~21-fold

increase in Kₘ for βG16BP and a similar Kₘ and Kᵢ for βG1P,

when compared with βPGMWT.

The mechanism of βPGM146A catalytic activity was explored by

preparing a TSA complex containing MgF₃⁻ and G6P29,45 and the

resulting βPGM146A:MgF₃:G6P TSA complex was investigated using

NMR spectroscopy. The observed
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19F chemical shifts are indistinguishable from those of the βPGMWT:MgF3:G6P TSA complex (Supplementary Fig. 9e, f) and a 1H15N-TROSY spectrum peak comparison (BMRB 7234) indicates an almost identical correspondence between frequencies. Such close agreement allowed a backbone resonance assignment (211 residues—100%) using 3D HNACAB and 3D HN(CA)CO spectra (BMRB 28097).

Residues with the largest chemical shift differences between the βPGM146A: MgF3:G6P and βPGMWT:MgF3:G6P TSA complexes are located within 4 Å of residue 146 (K145, A147 and A177) and within 5 Å of residue K145 (S48, V141 and A142) (Supplementary Fig. 4d). Taken together, these results confirm that βPGMWT can assume a stable and wild-type like βPGM146A:MgF3:G6P TSA complex in solution. The βPGM146A: MgF3:G6P TSA complex was crystallised and the structure was determined to 1.0 Å resolution (PDB 6YDJ; Supplementary Table 1). This complex superimposes very closely with the βPGMWT:MgF3:G6P TSA complex (non-H atom RMSD = 0.18 Å, PDB 2WF5) and identifies both the positioning of the K145 sidechain in the active site and the cis K145–A146 peptide bond (ω dihedral angle = 14°; compared with ω dihedral angle = 12° for the K145–P146 peptide bond in the βPGMWT:MgF3:G6P TSA complex) (Fig. 4b, d). The ε-phosphate group of G6P is in the distal site and the trinodal Mg2+ moiety mimicking the transferring phosphoryl group is coordinated in the proximal site between D8 (atom O41) and the 1-OH group of G6P. The donor–acceptor distance and the angle of alignment are 4.1 Å and 174°, respectively (compared with 4.3 Å and 176°, respectively for the βPGMWT:MgF3:G6P TSA complex). The catalytic Mg2+ ion coordination also has comparable octahedral geometry to the βPGMWT:MgF3:G6P TSA complex and to substrate-free βPGM146A. Together, these data demonstrate that βPGM146A is able to populate a cis K145–A146 peptide bond and achieve full domain closure with concomitant formation of transition state geometry. In addition, assuming that βG16BP binding is diffusion controlled, the increase in $k_{\text{cat}}$ for βG16BP in βPGM146A reflects the energetic cost of the trans to cis isomerisation of the K145–A146 peptide bond. As in βPGMWT, these results imply that conformer A of βPGM146A represents the more active form.

βPGM forms two different transient phospho-enzyme species. The possible involvement of conformer B in the modulation of enzyme activity was investigated using real-time NMR methods by comparing the phosphorylation of βPGM under saturating conditions of either F16BP (50–100 mM), AcP (60–100 mM) or βG16BP (35 mM). Residue A113 is a well-resolved reporter of the relevant species—conformer A and conformer B, and their phosphorylated counterparts, Aβ and Bβ. The carbonyl group of A113 is coordinated by the ε-amino group of K145 (in conformer A) or a water molecule (in conformer B) and its amide proton is hydrogen bonded to the carbonyl group of F7 (adjacent to the D8 nucleophile) (Fig. 4a, b).

On addition of F16BP to βPGM146A, the two dominant species observed are conformer B and Bβ (Fig. 2b). The presence of conformer B shows that the phosphorylation rate of βPGM146A is very similar to the dephosphorylation rate of Bβ (through hydrolysis), and only an apparent rate constant can be measured. The apparent rate constant for dephosphorylation was determined to be $0.003 \pm 0.00002$ s$^{-1}$ from the rate of reduction of the free F16BP concentration in 1H NMR experiments. The 1H and 15N chemical shifts of Bβ, assigned using fast acquisition 3D HNCO and 3D HNCA NMR experiments, mirror those of conformer B except for the active site residues F7–D8, A113–A115 and hinge residues T16–E18, owing to their proximity to phosphorylated D8 (Supplementary Fig. 4e). Resonances from the D137–A147 loop show no significant differences between both forms, indicating that the K145–A146 peptide bond is in a trans conformation in Bβ (the conformer B to Aβ transition results in large chemical shift changes for the D137–A147 loop; Supplementary Fig. 4f). Conformer B and Bβ are also observed when AcP was used as the phosphorylating agent, and a minor population of Aβ is present, correlating with a small increase in the population of Bβ relative to conformer B (Supplementary Fig. 7b). Identification of Aβ is based on the assignment and structure of the βPGMWT:BeF3 complex (BMRB 17851; PDB 2WF4), where the K145–P146 peptide bond is in a cis conformation and the K145 sidechain is engaged in the active site. Notably, when βG16BP was used as the phosphorylating agent, Bβ is not observed (Fig. 2b, Supplementary Fig. 7b). Instead, the Aβ:G6P and Bβ:G16BP complexes are the primarily populated species. The Aβ:G6P complex has similar chemical shifts to the βPGMWT:BeF3 complex, and the slow exchange between the Bβ:G16BP and the Aβ:G6P complexes correlates with the measured $k_{\text{cat}}$ values for βPGM146A.

In βPGMWT, Aβ is the dominant species observed on addition of F16BP (Fig. 2a, Supplementary Fig. 3c). Therefore, the phosphorylation rate of βPGMWT by F16BP under these conditions must be faster than the hydrolysis rate of Aβ ($k_{\text{cat,hydrolysis}} = 0.06 \pm 0.006$ s$^{-1}$). A minor population of the Aβ:G16BP complex is also present, indicating that the phosphorylation rate is slower than the chemical shift difference between the Aβ and Aβ:F16BP peaks (140 Hz). Significantly, a minor population of Bβ is also observed. This species is populated transiently (~5 min) and disappears at longer timeframes, whereas Aβ and the Aβ:F16BP complex populations remain dominant while the phosphorylating agent is at high concentration. Hence, the Bβ population is converging to the more stable Aβ species with a rate constant of $0.003 \pm 0.0003$ s$^{-1}$, which mirrors the trans to cis isomerisation rate constants of Xaa-Pro peptide bonds in model peptides. Equivalent behaviour is observed when AcP was used as the phosphorylating agent (Supplementary Figs. 3b, 7a), except that an AAcP complex is not detected. When βG16BP was used as a phosphorylating agent, Bβ does not accumulate at any point in the 3 h time course. The only detectable species is an Aβ:G16BP complex (Fig. 2a, Supplementary Figs. 3d, 7a), which is identified by the similarity of chemical shift distribution with the βPGM146A:G16BP complex (BMRB 27174; PDB 5OK1). The low intensity of the A113 peak, along with peaks of other active site residues (Fig. 2a, Supplementary Figs. 3d, 7a), results from millisecond conformational exchange between species within the catalytic cycle, which correlates with the measured $k_{\text{cat}}$ values for βPGMWT. Overall therefore, the consequence of phosphorylation by the reaction intermediate βG16BP is markedly different to that of other phosphorylating agents, in that it generates no detectable Bβ or lag phase, even when the initial population of conformer B is high.

Discussion

Substrate-free βPGMWT exists in solution as two distinct conformers with near-equal populations, which differ in the isomerisation state of the K145–P146 peptide bond and interconvert at a rate between 0.003 s$^{-1}$ and 1.0 s$^{-1}$. Conformer A contains the cis-isomer of this peptide bond, as observed in the crystal structures of substrate-free βPGMWT, whereas conformer B contains the trans-isomer, as mimicked by the βPGM146A variant. In the crystal structure of βPGM146A, the trans K145–A146 peptide bond positions the sidechain of K145 away from the site of phosphor transfer, which is significantly different to its location.
in all other substrate-free and TSA complex structures reported for βPGMWT. The removal of this positively charged amine group from the active site of conformer B disrupts the charge balance in the vicinity of the D8 nucleophile and therefore transition state stability will be severely impaired. However, kinetics data for the mutase reaction indicate that βPGMP146A is only ~20-fold less active than βPGMWT, and has a similar affinity for βG1P. In the crystal structure of the βPGMP146A:MgF3:G6P TSA complex, the K145–A146 peptide bond adopts the cis-isomer and the sidechain of K145 is able to engage in the active site. Hence, conformer A remains the preferred route for phosphoryl transfer in βPGMP146A, despite it being ~1000 times less stable than in βPGMWT because of the trans to cis isomerisation of the K145–A146 peptide bond.

This disruption of the active site has regulatory significance, as conformer B of βPGMWT constitutes a substantial population of the resting substrate-free enzyme. In vitro, linear initial kinetics and full activation of the enzyme are achieved only when βG16BP is used as the phosphorylating agent. Phosphorylation of both βPGMWT and βPGMP146A with either F16BP or AcP leads to a lag phase, with the lag caused by F16BP lasting ~3.5 times longer than that observed with AcP. Moreover, real-time NMR experiments establish that the phosphorylation of both βPGMWT and βPGMP146A with either F16BP or AcP result in the generation of B^p. In βPGMWT, B^p isomersises completely into A^p in <5 min, whereas in βPGMP146A, B^p is consistently more populated than A^p. In contrast, B^p is not observed for either βPGMWT or βPGMP146A when βG16BP is used as the phosphorylating agent. This result demonstrates that phosphorylation with βG16BP leads to the stabilisation of conformer A, resulting in production of A^p, regardless of the initial βPGM conformation, even for the βPGMP146A variant despite the considerable energetic cost of the conformer B to conformer A transition. Thus, conformer B and

![Fig. 6 Kinetic model of βPGM activity. a, b Reaction schemes for βPGMWT as conformer A or conformer B with different phosphorylating agents. The favoured pathways are shown (red text) for βPGMWT with A F16BP as a phosphorylating agent and b βG16BP as a phosphorylating agent. The βG16BP generating steps are highlighted in blue text. Fructose monophosphate (FMP) is either fructose 6-phosphate or fructose 1-phosphate. The complexes X:PG6P (X = A or B) and A:PG6IP denote explicitly the orientation of βG16BP bound in the active site. The double-headed arrows connecting A^p and B^p indicate that these species interconvert with a multi-second exchange rate, similar to that described for the interconversion of conformer A and conformer B.](image)

![Fig. 7 Mechanisms of regulation and activity profiles in monomeric enzymes. In allostery, binding (or reaction) of an allosteric effector (purple rectangle) outside of the active site shifts the enzyme population from an inactive form (red circle and red profile) to an active form (green square and green profile), which stimulates the transformation of substrate (blue oval) to product (yellow triangle) at the catalytic rate (k_cat, green arrow). In allokairy, binding of substrate in the active site shifts the enzyme population from an inactive form to an active form, at an exchange rate (k_ex) that is similar to k_cat, resulting in time-dependent activity profiles (gradient of light green to dark green profiles). Following exhaustion of substrate, the enzyme population returns to the original equilibrium position. In allomorphy, reaction of the activating substrate, termed here allomorphic full activator (green hexagon), in the active site shifts the enzyme population from an inactive form to an active form, which stimulates the transformation of the native substrate (blue oval) to product (yellow triangle) at the maximal catalytic rate (k_cat, green arrow and green profile). However, reaction of alternatives substrates, termed here allomorphic partial activators (pink pentagon), in the active site are unable to shift the enzyme population from an inactive form to an active form, resulting in a slower overall catalytic rate (k_ex, pink arrow and pink profile). The exchange rate (k_ex) between the two enzyme forms is much slower than k_cat. Following exhaustion of the allomorphic activator, the enzyme population returns to the original equilibrium position.](image)
βP are significantly less-active forms of βPGM, and the slow transition from BβP to AβP is part of the characteristic lag phase observed in the coupled assay kinetics. The longer lag in the F16BP experiments and the observation of a βPGMWT:F16BP complex are consistent with F16BP having a slower phosphorylation rate than AcP. Therefore, βPGM is able to follow alternative kinetic pathways depending on the phosphorylating agent present, with its overall catalytic rate determined by the rate-limiting step in each pathway (Fig. 6a, b).

The response of βPGM to different phosphorylating agents also has functional significance. In L. lactis, the sole source of βG16BP is βPGM itself. In contrast, F16BP accumulates during glycolysis, reaching a concentration of up to ~50 mM7. Hence, F16BP is likely to be the primary source of βPGM activation in vivo, with AcP contributing to a lesser extent53,43. Correspondingly, a proportion of βPGM will populate the less-active BβP species. Following a switch from glucose to maltose or trehalose metabolism, which will greatly increase the βG1P concentration (Supplementary Fig. 1), βG16BP will begin to accumulate (Fig. 1, 6a, b). As a result, the conformer B and BβP populations will be recruited into the more active AβP species. This two-state control mechanism allows L. lactis to effectively catabolise maltose or trehalose, while the increase in transcription of g6pB is in progress30. Furthermore, when carbohydrate levels are low, a significant proportion of the basal βPGM population will be maintained as conformer B, which will consequently reduce the undesirable dephosphorylation of F16BP and also hinder the conversion of G6P to G1P.

The multi-second substrate-dependent non-allosteric conformational exchange mediated through cis–trans proline isomerisation seen in βPGM represents a mechanism of post-translational enzyme regulation. This regulation mechanism relies on the existence of alternative pathways with different rate-limiting steps, where the catalytic rate depends on the capacity of an activator, acting as a substrate, to bias the enzyme population towards the fastest pathway, by stabilising the most active conformation (Fig. 7). Similar to allostery and alloclarity, this mechanism depends on the ability of the enzyme to adopt at least two conformations with distinct activities, but does not require the binding of an additional effector to the protein, nor an equivalence between the conformational exchange rate and the catalytic rate. We suggest the name allomorphy for this mechanism, from the greek allos meaning other and morphē meaning form, in keeping with the previously described allosteroy and alloclarity mechanisms15.

Allomorphy may modulate the activity of other monomeric enzymes with hysteretic behaviour, i.e., those that exhibit a burst or lag phase in their kinetic profile19. Several theoretical models have been put forward to rationalise hysteretic behaviour, such as the mnemonic50 and the ligand-induced slow transition51 models, but detailed structural-based molecular mechanisms have proved elusive. To our knowledge, only one such mechanism, alloclarity in human glucokinase, has been described in detail15,16. Alloclarity is a different fine control regulatory mechanism and is potentially widespread, at least across phosphomutases; for example, both rabbit muscle and L. lactis α-phosphoglucomutases appear to be hysteretic enzymes32,33, but belong to very different protein superfamilies. Like βPGM, these enzymes require a phosphorylating agent to initiate the catalytic cycle and, for the latter, the use of the reaction intermediate results in linear kinetics, whereas alternative phosphorylating agents produce a lag phase in their kinetic profiles. Similarly, α-phosphomannomutase from Galdieria sulphuraria, which also requires the addition of a phosphorylating agent to initiate the catalytic cycle, exhibits linear kinetics when α-mannose 1-phosphate and α-mannose 1,6-bisphosphate (or α-glucose 1-phosphate and αG16BP) are included in the reaction, but has a lag phase when there is a mismatch between substrate and phosphorylating agent, or when F16BP is used as the phosphorylating agent44. All of these observations are consistent with the presence of alloclary control.

In summary, allomorphy is a fine control mechanism by which part of an enzyme population is maintained in a more latent state, and is quickly switchable when high and low activity levels, without allosteric effectors. It delivers important control with which L. lactis is able to reconcile two seemingly contradictory requirements: the need to maximise its responsiveness to changes in carbohydrate source and the need to minimise unproductive diversion of valuable metabolites.

Methods

β-phosphoglucomutase (βPGM) expression and purification. Wild-type βPGM (βPGMWT) and the F146A variant (βPGMWT,F146A) proteins were expressed using either 15N or 1H15N isotopic enrichment65 and purified using metalochromatography that minimised the presence of contaminating phospholipid transfer enzymes (e.g., phosphoglucone isomerase and βPGM from E. coli)31,42. The βPGMWT and βPGMWT,F146A genes were cloned into the PET-22b(+) plasmid, transformed into E. coli strain BL21(DE3) cells and expressed in identically labelled minimal media. The cells were grown at 37 °C with shaking until OD600 = 0.6, then cooled at 25 °C and induced with 0.5 mM isopropyl β-D-1-thiogalactopyranoside for a further 18 h. Cells were harvested by centrifugation at 10,000 rpm for 10 min. The cell pellet was resuspended in ice-cold lysis buffer (50 mM K+ HEPES (pH 7.2), 2 mM NaN3, 1 mM EDTA) supplemented with complete protease inhibitor cocktail and lysed by six cycles of sonication. The cell lysate was cleared by centrifugation at 20,000 rpm for 35 min at 4 °C. The supernatant was filtered using a 0.22 μm syringe filter and loaded onto a DEAE-Sepharose fast flow anion-exchange column. Proteins bound to the column were eluted with a gradient of 0–50% lysis buffer containing 1 M NaCl. Fractions containing βPGM were purified further using a HiLoad 16/60 Superdex 75 size-exclusion column previously equilibrated with lysis buffer containing 1 M NaCl. Final fractions were pooled, exchanged into 50 mM K+ HEPES buffer (pH 7.2) containing 2 mM NaN3 and concentrated to ~1.6 Mm for storage at ~20 °C.

Reagents. Unless otherwise stated, reagents were purchased from Sigma-Aldrich, GE Healthcare, Mellford Laboratories or CourtexNet. βG1P was synthesised enzymatically from maltose using maltose phosphorylase (EC 2.4.1.8)31. A solution of maltose (600 mM) was incubated overnight with 1.2 units ml−1 maltose phosphorylase in 0.5 M sodium phosphate buffer (pH 7.0) at 30 °C and βG1P production was confirmed using 31P NMR spectroscopy. Maltose phosphorylase was removed using a Vivapsin (5 kDa MWCO) and the resulting flow-through was used without further purification. βG16BP was produced enzymatically from βG1P and the D170N variant of βPGM (βPGMWT,D170N) was expressed using methodology as detailed above30. βG1P and AcP were incubated with βPGMWT,D170N for 4 h at 25 °C and the reaction was quenched by heating at 90 °C for 10 min. βG16BP was purified using barium salt precipitation.

NMR spectroscopy. 1H15N-TROSY NMR spectra of βPGMWT and βPGMWT,F146A were acquired at 298 K using 0.5–1 mM 1H15N-βPGM in standard NMR buffer (50 mM K+ HEPES (pH 7.2), 5 mM MgCl2, 2 mM NaN3 with 10% (v/v)2H2O and 1 mM TSP). Typically, 1H15N-TROSY spectra were accumulations of 16 transients, with 256 increments and spectral widths of 32–36 ppm centred at 120 ppm in the indirect 1H dimension. 1H15N-TROSY-based ZZ-exchange experiments were performed at mixing times of 100, 300, 500 and 900 ms. Rapid acquisition 1H–15N BEST-TROSY experiments to monitor the steady-state behaviour of 1H15N-βPGMWT (0.2 mM) and 1H15N-βPGMWT,F146A (0.2 mM) were acquired in standard kinetic buffer (50 mM K+ HEPES (pH 7.2), 5 mM MgCl2, 2 mM NaN3, with 10% (v/v) 2H2O and 1 mM TSP) containing either 50–100 mM F16BP, 60–100 mM AcP or 35 mM βG16BP. The 1H15N-BEST-TROSY spectra were recorded at 298 K using a Bruker 600 MHz Neo spectrometer equipped with a 5-mm TCI cryoprobe and a 11 mm experiments (16 transients, 128 increments and a recycle delay of 0.2 s) with selective 1H pulses centred on the amide axis (8.3 ppm). Excitation pulses (90°) were 1.7 ms (pulse shape Eburp2), whereas refocusing pulses (180°) were 1.4 ms (pulse shape Reburp). The experimental dead-time was ~5 min.

For βPGMWT,F146A prepared in standard kinetic buffer containing 50 mM F16BP, βG16BP dephosphorylation was monitored at 298 K by consecutive one-dimensional 1H NMR experiments recorded with 16 transients, a 1 s recycle delay and a spectral width of 32 ppm centred on the water signal. Following 0.3 Hz Lorentzian apodisation and baseline correction, normalised integral values of the F16BP peak (4.22–4.15 ppm) were plotted against time to give a kinetic profile. The initial linear steady-state portion of the kinetic profile was fitted using an linear least-squares fitting algorithm included in MATLAB 2018a to derive an apparent dephosphorylation rate constant.
To observe the species present immediately following the addition of phosphorylating agent to $\beta$PGMWT, NMR experiments were recorded using the use of a home-built rapid mixing device. The equipment comprised a 2 ml internal diameter ETEF tube (GE Healthcare), connected at one end to a 1 ml syringe and inserted at the other end through the lid of an NMR tube. The tube was loaded with phosphorylating agent (550 µl 100 mM F16BP or 250 µl 320 mM Acp, prepared in standard kinetic buffer) and a small, additional volume of water was drawn in to prevent premature mixing of the phosphorylating agent with the 550 µl 1.2 mM $^{13}$N-$\beta$PGMWT sample prepared in standard kinetic buffer. The rapid mixing device was loaded into a Bruker 600 MHz Neo spectrometer and allowed to equilibrate thermally at 298 K. Following mixing by syringe action of the phosphorylated protein, $^1$H and $^{15}$N resonances were recorded by 2H, 1H, and 3C backbone resonance assignment of $^{13}$C$^{15}$N$^{13}$C-$\beta$PGMWT in standard NMR buffer containing 10 mM tris were acquired at 298 K on a Bruker 800 MHz Avance III spectrometer equipped with a 5-mm TXI cryoprobe and $^1$H axis gradients. The standard Bruker suite of $^1$H$^3$N$^2$TROSY and $^3$D TROSY-based constant time experiments were acquired (HONCO, HNCACO, HN(CCA)C, HNCCAB) using non-uniform sampling (NUS) with a multi-dimensional Poisson Gap scheduling strategy with exponential weighting. NUS data were reconstructed using multi-dimensional decomposition in TopSpin38. Backbone resonance assignments for conformer A and conformer B present simultaneously in the spectra were obtained using a simulated annealing algorithm employed by the Autodesk NMR suite and assignments were confirmed using backbone amide to amide correlations obtained from TROSY-based (H)$\text{CoA}$ (NHC) and H(NHCOA)(NH) experiments.59 Multi-dimensional heteronuclear NMR spectra for $^1$H, $^{13}$N and $^{15}$C backbone resonance assignment of the $^{13}$C$^{15}$N$^{13}$C-$\beta$PGMWT$_{\text{MgF}_6}\text{G6P}$ TSA complex in standard NMR buffer containing 10 mM G6P were acquired at 298 K on a Bruker 800 MHz Avance l spectrometer equipped with a 5-mm TXI probe and $^1$H axis gradients. $^1$H$^3$N$^2$TROSY and $^3$D TROSY-based constant time experiments were acquired (HNCACO) and HNCCAB) and resonance assignments were obtained using astools55. Multi-dimensional heteronuclear NMR spectra for $^1$H, $^{15}$N and $^{13}$C backbone resonance assignment of phosphorylated $^{13}$C$^{15}$N$^{13}$C-$\beta$PGMWT$_{\text{MgF}_6}$ in standard kinetic buffer containing 75–100 mM F16BP were acquired at 298 K on a Bruker 800 MHz Avance l spectrometer equipped with a 5-mm TXI probe and $^1$H axis gradients. $^1$H$^3$N$^2$TROSY and $^3$D TROSY-based constant time experiments were acquired (HNCACO) and HNCCAB) and resonance assignments were obtained using astools55. NUS data were reconstructed using multi-dimensional decomposition in TopSpin38. TROSY resonances were assigned by comparing the correlated $^{13}$C chemical shifts with those of $\beta$PGM$_{\text{wt}}$ (BMRR 279204) and the $\beta$PGMWT$\cdot$BeF$_3$ complex (BMRR 178513). Experiments were processed using TopSpin (Bruker) or FEILIX (Felix NMR, Inc.) and NMR figures were prepared using FELIX. $^1$H chemical shifts were referenced relative to the internal TSP signal resonating at 0.0 ppm. $^{13}$C and $^{15}$N chemical shifts were referenced using the nuclear-specific gyromagnetic ratios. Differences in chemical shift were calculated as: 
\[ \Delta \delta = (\delta_{\text{sample}} - \delta_{\text{Ref}}) + \left((0.12 \times (\delta_{\text{sample}} - \delta_{\text{Ref}})) / 12\right) \]
where X and Y are the two species being compared.

Reactivity kinetics for $\beta$PGMWT-catalysed reactions were followed using a Bruker 500 MHz DRX spectrometer (operating at 202.456 MHz for $^1$H) equipped with a room-temperature broadband probe. The equilibration of 10 mM $\beta$GIP with G6P by 1–3 mM $\beta$PGM$_{\text{wt}}$ was measured in standard kinetic buffer at 298 K. The reaction was initiated by and timed from the addition of 20 mM AcP and monitored by the acquisition of consecutive $^{31}$P spectra without proton-axis gradients. Samples were prepared using a homemade rapid mixing device. The equipment comprised a 2 m length of 0.8 mm i.d. PTFE tubing with a 5-µm flow rate. The solution was added to the 4000, 200 mM sodium acetate and 100 mM tris-HCl (pH 7.5)). Crystals were grown at 290 K by hanging-drop vapour diffusion using a 2 µl drop suspended on a 4000, 200 mM sodium acetate and 100 mM tris-HCl (pH 7.5). Crystals were grown at 290 K by hanging-drop vapour diffusion using a 2 µl drop suspended on a 4 m-water drop (2, 5, 10, 35, 50, 100 µM). The initial rate of G6P production was measured indirectly using a glucose 6-phosphate dehydrogenase (G6PDH) coupled assay, in which G6P is oxidised and concomitant NAD$^+$ reduction is monitored by the increase in absorbance at 340 nm (NAD$^+$ extinction coefficient = 6220 M$^{-1}$ cm$^{-1}$). The rate of G6P production was measured using a linear least-squares fitting algorithm to determine the reaction velocity ($v$) at each $\beta$GIP and $\beta$G16BP concentration at a total enzyme concentration ($E_\text{total}$). Mean data from triplicate measurements were subsequently globally fitted to Eq. 128, which is derived for a ping-pong mechanism and adapted to account for $\beta$GIP inhibition (K) to calculate $K_{\text{cat}}$ and individual $K_v$ values for $\beta$PGMWT and $\beta$PGMP146A, with their corresponding standard deviations, using an in-house python non-linear least-squares fitting program.

\[ v = \frac{k_{\text{cat}}[E_\text{total}][\beta\text{GIP}][\beta\text{G16BP} + K_{\text{G16BP}}]}{K_{\text{cat}}[E_\text{total}][\beta\text{GIP}] + K_{\beta\text{GIP}}[\beta\text{G16BP}] + K_{\beta\text{GIP}}[\beta\text{GIP}] + K_{\beta\text{GIP}}[\beta\text{G16BP}]} \]

Kinetik experiments demonstrating the effect of different phosphorylating agents were conducted by the addition of either 5 nM $\beta$PGMWT or 200 nM $\beta$PGMP146A to solutions containing either 1 mM F16BP, 8 mM Acp or 10 µM G16BP, together with 1 mM NAD$^+$, 5 units ml$^{-1}$ G6PDH and 50 µM G16BP. F16BP represents an equilibrium mixture of an a-anomer (15%), a b-anomer (81%) and two open chain forms with an interconversion rate of 8 ± 1 s$^{-1}$.

### X-ray crystallography

For the crystallisation experiments of $\beta$PGMWT, $\beta$PGMP146A the $\beta$PGMP$_{\text{MgF}_6}\text{G6P}$ TSA complex and the $\beta$PGMP$_{\text{MgF}_6}\text{G6P}$ TSA complex, frozen aliquots of $\beta$PGMWT or $\beta$PGMP146A were prepared in standard kinetic buffer to a final concentration of 1 nM $\beta$GIP$\cdot$G16BP (2, 5, 10, 35, 50, 100 µM). The initial rate of G6P production was measured by the increase in absorbance at 340 nm (NAD$^+$ extinction coefficient = 6220 M$^{-1}$ cm$^{-1}$). The crystals were harvested using a mounted Litho-Mop Loopy loop (Molecular Dimensions Ltd) and were cryo-protected in their mother liquor containing an additional 25% (v/v) ethylene glycol (40% and 50 mM citrate for the $\beta$PGMWT$c$-citrate and $\beta$PGMP146A$c$-citrate crystals) prior to plugging into liquid nitrogen. Diffraction data were collected at 100 K on the MX beamlines at the Diamond Light Source (DLS), Oxfordshire, United Kingdom.

Data were processed using the xia2 pipeline61 and resolution cutoffs were applied using CC-half values and Aimless62. The crystals diffracted in the P212121 space group with unit cell parameters $a = 94.22$, $b = 94.22$, $c = 100.0$ Å, $\beta = 90$°. Data were scaled using the bg3 algorithm. The structure was solved by molecular replacement with MolRep and structures were refined to 2.6 Å resolution using the ABInitio software suite63. Overall, the structure of $\beta$PGMWT$c$-citrate is similar to the $\beta$PGMP146A$c$-citrate structures determined at 2.6 Å resolution, with the main differences being in the relative positioning of the Mg$^{2+}$ ion and the citrate ligand. The Mg$^{2+}$ ion is located in a deeper pocket than in the $\beta$PGMP146A$c$-citrate structure, which is likely to be due to the difference in the positioning of the citrate ligand.
figures were prepared using PyMOL (The PyMOL Molecular Graphics System, version 1.8, Schrödinger, LLC). To confirm the isomerisation state of the K145–A146 peptide bond in the structures of substrate-free βPGMwt and the βPGMP146A:MgF3:G6P TSA complex, difference density maps (Fo–Fc) were generated using REFMAC5 with the SI44–P148 segment omitted from the final structures. Omit map figures were prepared using CCP4mg (version 2.10.9)\textsuperscript{12}. Additional details for X-ray crystallography data collection, data processing and refinement are provided in Supplementary Table 1.

Data availability

Data supporting the findings of this manuscript is available from the corresponding author upon reasonable request. The atomic coordinates and structure factors have been deposited in the Protein Data Bank (www.rcsb.org) with the following codes: βPGMwt-citrate complex (PDB 6DYM), substrate-free βPGMwt (PDB 6YDL), substrate-free βPGMP146A (PDB 6YD4) and βPGMP146A:MgF3:G6P TSA complex (PDB 6YD1). The NMR chemical shifts have been deposited in the BioMagResBank (www.bmrbr.wisc.edu) with the following accession numbers: substrate-free βPGMP146A conformer A (BMRBR 28095), substrate-free βPGMP146A conformer B (BMRBR 28096) and βPGMP146A:MgF3:G6P TSA complex (BMRBR 28097).

Code availability

Code developed in Python3 and bash for this study is publicly available under an MIT license and can be found on GitHub [https://doi.org/10.5281/zenodo.4022248].

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H.P.W., F.A.C.N., N.J.B., C.R.T., A.J.R. performed research study; H.P.W., F.A.C.N., C.R.T., A.J.R. performed and analysed X-ray crystallography experiments; F.A.C.N., C.R.T., A.J.R. performed and analysed X-ray crystallography experiments; F.A.C.N. conceived and developed the allomorphy mechanism; H.P.W., F.A.C.N., N.J.B., C.R.T., J.P.W. wrote the paper with help from all authors.

Competing interests
The authors declare no competing interest.

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