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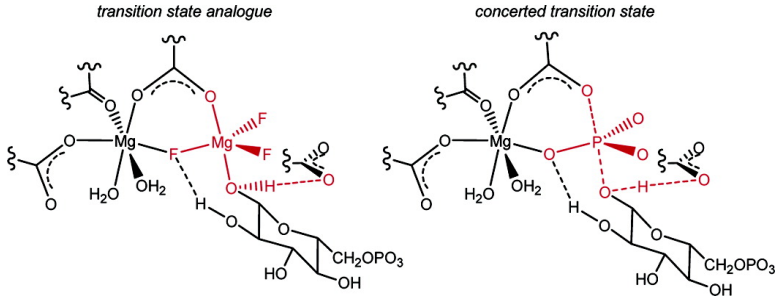


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# High-Energy Intermediate or Stable Transition State Analogue: Theoretical Perspective of the Active Site and Mechanism of β-Phosphoglucomutase

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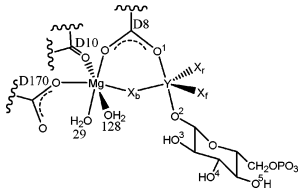
A recent crystal structure[1] of β-phosphoglucomutase[2] (PGM) from *Lactococcus lactis* was refined as a five-coordinate phosphorus (with five oxygen ligands), which the authors suggested is a "high-energy reaction intermediate" for phosphoryl transfer in the isomerization of β-glucose 1-phosphate (β-G1P) to β-glucose 6-phosphate (β-G6P). Subsequently, it was suggested that this structure is actually a transition state analogue with a five-coordinate magnesium with two oxygen and three fluorine ligands.[3] The active site of PGM (depicted in Scheme 1) has a five-coordinate atom (Y = P or Mg) and a pseudo-octahedral Mg, coordinated by four terminal ligands (two waters, one carboxylate oxygen from ASP170, and one backbone carboxyate oxygen from ASP10) and two bridging ligands (one of the carboxylate oxygens from ASP8 and an atom, $X_b$, bridging to the five-coordinate species, Y).

***Scheme 1.*** Diagrammatic Representation of the PGM Active Site




This work[4] utilizes two layered ONIOM(B3LYP:PM3MM) calculations to address the nature of the PGM active site structure and clarify the identity of this five-coordinate atom, Y, and its ligation.

The model employed in the current study is based on the active site of the PGM enzyme (Scheme 1) with the 30 surrounding residues (103 B3LYP atoms, 390 total atoms). The phosphoglucose and the neighboring residues were freely optimized using DFT, while the outer residues were constrained in space at their crystallographically determined locations with energy calculations using PM3MM (see the Supporting Information for full details).

There is considerable hydrogen bonding to the active site, specifically from neighboring residues to the $YX_3O_2$ unit on the right side of the view in Scheme 1 (see Figure 1 and Supporting Information, Table S1 gives a list of the hydrogen bonding to the active site).

Calculations were performed with both $MgF_3$ and $PO_3$ for $YX_3$ starting at the coordinates of the crystal structure. With $YX_3$ = $MgF_3$, one minimum is located with a stable five-coordinate magnesium (Figure 1). This structure is consistent with the previous proposal[3] of a transition state (TS) analogue containing $MgF_3$ in the active site, and the geometry matches that of the reported crystal quite well (Scheme 2). An extensive hydrogen-bonding network present around the right side of the active site stabilizes the TS analogue. The Mg on the right has three equatorial F and two axial O (Figure 1 and Scheme 2). While the bridging fluorine, $F_{b(ridge)}$, is hydrogen bonded to one OH of the bound glucose, the two non-bridging fluorines, $F_{r(ear)}$ and $F_{f(ront)}$, are hydrogen bonded to several residues. $F_r$ interacts with an OH group (SER114) and two backbone

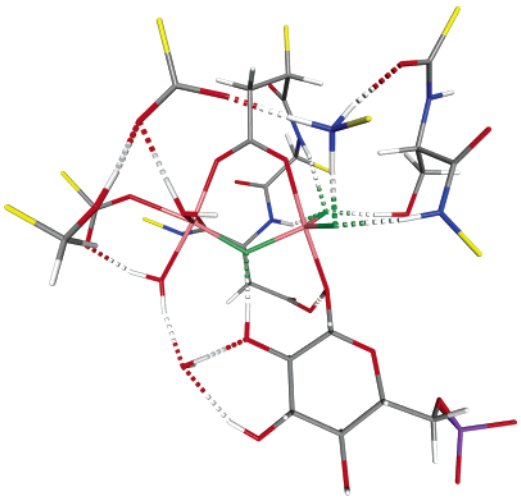
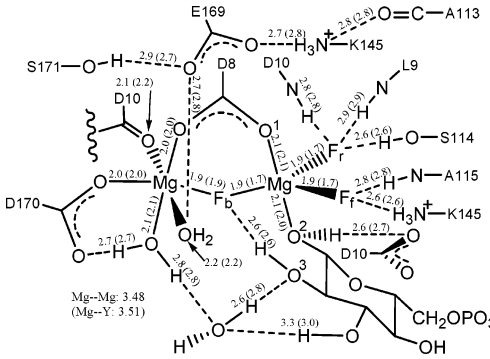


***Figure 1.*** A truncated view of the ONIOM(B3LYP:PM3MM) optimized geometry of the PGM active site when $YX_3$ = $MgF_3$. The yellow atoms and the 6-phospho group are calculated with PM3MM; only the yellow atoms are constrained to their crystallographic locations. The remaining atoms are freely optimized with B3LYP. This five-coordinate trigonal bipyramidal magnesium is a transition state analogue that is stabilized by significant hydrogen bonding from neighboring amino acid residues.

***Scheme 2.*** Hydrogen Bonding with Various Neighboring Residues Stabilizes This "$MgF_3O_2$" Transition State Analogue[a]




[a] Calculated and experimental (in parentheses) distances are given between heavy atoms in angstroms. ASP10 is particularly critical to the structure.

imido NH groups (LEU9 and ASP10); and $F_f$ interacts with one $RNH_3^+$ group (LYS145) and one backbone imido NH group (ALA115). Critical to the stabilization of this TS analogue, a protonated carboxylic acid group (ASP10) donates a proton to the glucose oxygen (labeled $O^2$) in the lower axial position. The second axial ligand ($O^1$) is the bridging carboxylic acid group of ASP8.

However, with $YX_3$ = $PO_3$, the optimization of five-coordinate phosphorus does not converge to a stable minimum but to a transition state (TS) for phosphoryl transfer. This moderate barrier TS (+14.0 kcal $mol^{-1}$, 147*i* $cm^{-1}$) connects the reactant (0 kcal $mol^{-1}$, hydrogen bound glucose 6-phosphate) to a slightly less stable



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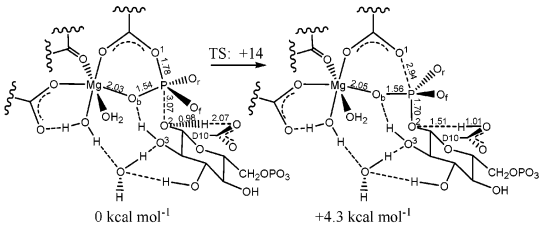
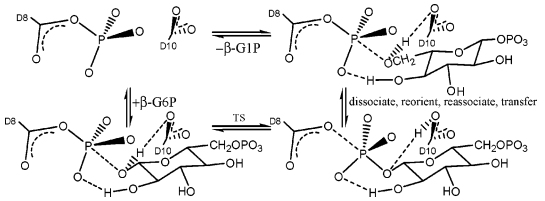
***Scheme 3.*** Schematic of When $YX_3 = PO_3$, a Tetrahedral Phosphorus Reactant, Transfers the Phosphoryl Group in a Concerted Five-Coordinate Phosphorus Transition State (with concomitant proton transfer from glucose to ASP10) To Form the Glucose 1,6-Bis-phosphate Intermediate[a]




[a] Calculated distances are given in angstroms.

***Scheme 4.*** Isomerization of $\beta$-G6P to $\beta$-G1P




intermediate (+4.3 kcal mol$^{-1}$, hydrogen bound glucose 1,6-bis-phosphate) (see Schemes 3 and 4). The TS is concerted[5] ($A_ND_N$,[6] i.e., it has no phosphorane intermediate) with "five-coordinate" phosphorus and has a concomitant proton transfer from the hydroxyl group of glucose to ASP10.[7] The reactant has (1) a shorter P−O$^1$ bond length (1.78 Å) as compared with those of the TS (2.24 Å) and the bis-phospho-intermediate (2.94 Å); (2) a longer P−O$^2$ bond length (3.07 Å) as compared with those of the TS (1.99 Å) and the intermediate (1.70 Å); and (3) a shorter glucose O$^2$−H bond (0.98 Å) than those of the TS (1.05 Å) and the intermediate (1.51 Å). The classification of the TS (associative or dissociative) is complicated by the fact that it has this simultaneous proton transfer. The TS has bond breaking character for the P−O$^1$ ($\Delta = +0.46$ Å) and O$^2$−H ($\Delta = +0.07$ Å) bonds and bond making character for the P−O$^2$ bond ($\Delta = +1.08$ Å); therefore, one might classify this TS as associative. In any case, the concerted transfer of the phosphoryl group is clear; there is no five-coordinate phosphorane or three-coordinate metaphosphate intermediate.

These structures are connected with the sequence of events that lead to glucose isomerization[1a] (Scheme 4): imagine starting with the resting state (phosphorylated) enzyme, binding and phosphorylating $\beta$-G6P to form the bis-phospho-intermediate ($\beta$-G1,6-diP). $\beta$-G1,6-diP could then dissociate, reorient, and reassociate, but with 1-$PO_3$ oriented toward the active site. The active site could then dephosphorylate $\beta$-G1,6-diP and $\beta$-G1P could dissociate, which completes the isomerization. How does the "$MgF_3$" transition state analogue form (and persist, since fluoride has not been measured as an inhibitor)? After the dissociation of $\beta$-G1,6-diP, ASP10 could deprotonate, adventitious $Mg^{2+}$ and $F^-$ (from the $NH_4F$ buffer) could bind to the active site, and $\beta$-G6P could then bind, forming the $MgF_3$ structure. Perhaps $MgF_3$ is a very weakly bound active site competitor, stabilized by hydrogen bonding, and persists only at the low temperature utilized during the crystallography. Alternatively, the reported structure could be an admixture of bound $\beta$-G6P and $\beta$-G1,6-diP.

Both the TS analogue (when $YX_3 = MgF_3$) and TS (when $YX_3 = PO_3$) are stabilized by the extensive hydrogen-bonding network around the active site, and this network is essential to the active site structure and function. One can easily see the importance of the conserved ASP10,[2a,8] for it not only provides key stabilization to the active site but also acts as a proton donor/acceptor to the glucose oxygen during the phosphoryl transfer when $YX_3 = PO_3$. Site-directed mutagenesis studies could establish the vital role of the conserved ASP10 residue as a proton acceptor/donor for the OH group of glucose.[7]

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**Supporting Information Available:** Details of the models examined in the current study, including amino acid residue labels and constraints; four anaglyphs: one of the optimized five-coordinate magnesium TS analogue ($MgF_3 = YX_3$) and one each of the three optimized phosphorus species ($PO_3 = YX_3$); and animations of the transition state and its imaginary mode (GIF). The anaglyphs represent "3-D views" of the hydrogen-bonding interactions around the active site. This material is available free of charge via the Internet at http://pubs.acs.org.

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(5) Care must be taken when classifying reaction mechanisms, particularly phosphoryl transfer reactions.[6b] There has been some confusion in the use of the terms dissociative and associative in the enzymology literature. The associative character of this TS does not lead to a pentavalent intermediate. In organic nomenclature, this transition state could be termed an $S_N2$-like process. In inorganic nomenclature, this transition state could be termed an interchange associative ($I_a$)-like process.

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