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# $MgF_3^-$ and α-Galactose 1-Phosphate in the Active Site of β-Phosphoglucomutase Form a Transition State Analogue of Phosphoryl Transfer

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Enzyme catalyzed phosphoryl transfer reactions play integral roles in metabolism, regulation, and cell signaling and are associated with the largest enzymatic rate enhancements yet known.[1] Trifluoromagnesate ($MgF_3^-$) has recently been identified as a trigonal planar mimic for the $PO_3^-$ group in transition states (TS) for several of these enzymes.[2] Its occurrence is likely to be even more widespread since transition state analogue (TSA) structures previously thought to contain $AlF_3$ most likely instead contain $MgF_3^-$.[2d] Using β-phosphoglucomutase (β-PGM), which catalyzes the interconversion of β-glucose 1-phosphate (βG1P) to β-glucose 6-phosphate (G6P) involving a transient phosphoenzyme and a β-glucose 1,6-bisphosphate (βG16BP) intermediate, we have shown previously the formation of a stable $MgF_3^-$ TSA complex with G6P (PGM-$MgF_3$-G6P-TSA),[2c,f] and the analogous PGM-$MgF_3$-βG1P-TSA complex has been characterized kinetically.[2e]

The reality of formation of PGM-$MgF_3$-TSA complexes, and by implication in other phosphoryl transfer enzymes, has been questioned.[3] The major piece of evidence against the stabilization of $MgF_3^-$ within a protein scaffold, resulting in a readily observable population of a TSA complex, stems from the behavior of β-PGM with α-galactose 1-phosphate (αGal1P). In the absence of fluoride, this sugar phosphate forms a complex (PGM-αGal1P, $K_i = 30\ \mu M$) having a crystal structure[3d] with the cap and the core domains closed together and with three ordered water molecules occupying positions analogous to those of the three fluorine atoms in the PGM-$MgF_3$-G6P-TSA complex.[2f] It was argued "The β-PGM-α-Gal1P complex provides the ideal binding partner for $MgF_3^-$ if such a chemical species were to form from $MgCl_2$ and $NH_4F$." However, PGM-αGal1P crystals with and without fluoride proved to have indistinguishable structures, showed no $MgF_3^-$ in the catalytic site, and were used as standards for a Bradford protein assay and Malachite Green phosphate assay to provide "Direct evidence for the β-PGM-β-D-glucose-6-phosphate-1-phosphorane structure..."[3d] We now resolve this controversy by showing that β-PGM does indeed populate a PGM-$MgF_3$-αGal1P-TSA complex as the overwhelmingly predominant species in solution. The coordination of the trigonal planar $MgF_3^-$ moiety by αGal1P and β-PGM is very close to that observed in the PGM-$MgF_3$-G6P-TSA complex.

An overlay of the PGM-$MgF_3$-G6P-TSA complex (2wf5)[2f] with the PGM-αGal1P complex in the absence of fluoride (1z4o)[3d] gives a remarkable level of agreement, with rms deviations of 0.38 Å for all main-chain atoms and 0.45 Å for the three ordered water molecules and the three fluorine atoms in the PGM-$MgF_3$-G6P-TSA complex (Figure 1), despite changes in the sugar orientation. G6P in the PGM-$MgF_3$-G6P-TSA complex has its C(6)-phosphate group in the distal binding pocket and the C(1)-β-hydroxyl group axially coordinates the $MgF_3^-$ moiety (together with the D8 carboxylate group), whereas αGal1P in the PGM-αGal1P complex has its C(6)-hydroxyl group close to D8.

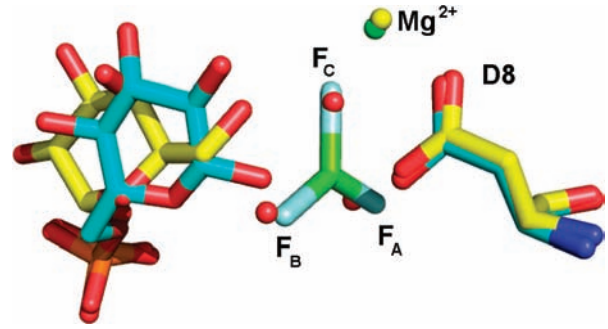


***Figure 1.*** Active site regions of PGM-αGal1P (yellow) and PGM-$MgF_3$-G6P-TSA (cyan) complexes. G6P coordinates the $MgF_3^-$ moiety (green + gray) by the C(1)-β-hydroxyl group. The three ordered water molecules (red spheres) in the PGM-αGal1P complex are shown. The phosphate groups (orange + red) occupy the same distal binding pocket.

The identification of enzyme complexes in solution that contain $MgF_3^-$ is readily achieved using solution NMR methods.[2c,d,f] To investigate whether a PGM-$MgF_3$-αGal1P-TSA complex forms, 10 mM $NH_4F$ was added to a solution containing 0.5 mM β-PGM, 5 mM $MgCl_2$, and 50 mM αGal1P at pH 7.2. The resulting $^{19}F$ NMR spectrum (Figure 2) indeed showed three intense fluorine resonances, which displayed a 1:1:1 ratio and a 1:1 stoichiometry with protein concentration, as expected for the formation of a PGM-$MgF_3$-αGal1P-TSA complex. The chemical shifts of the three fluorine nuclei fall in the range previously observed for $MgF_3^-$ species.[2d] The location of the three fluorine atoms in the active site was established using frequency-selective $^{19}F-^1H$ NOEs between the three fluorine resonances ($F_A$, $F_B$, and $F_C$) and backbone amide protons from residues L9, D10, A115, and S116 of β-PGM, as demonstrated previously for both the PGM-$MgF_3$-G6P-TSA complex[2c] and the PGM-$AlF_4$-G6P-TSA complex.[2d] In addition, strong $^{19}F-^1H$ NOEs were observed between fluorine resonances and the $^1H$ resonances of the C(6)-methylene group of αGal1P, confirming the close proximity of the $MgF_3^-$ moiety and the C(6)-$CH_2OH$ group. Scalar couplings associated with the F···H−N hydrogen bonds for the amide groups of D10 ($J_{FH} = 41$ Hz, $J_{FN} = 26$ Hz) and A115 ($J_{FH} = 44$ Hz, $J_{FN} = 21$ Hz) were also

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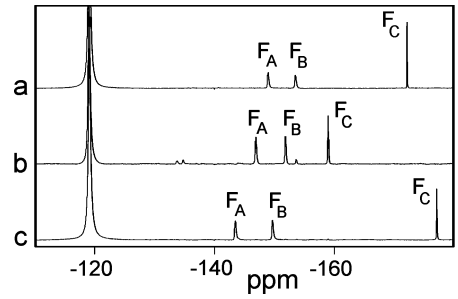


***Figure 2.*** $^{19}$F NMR spectra of PGM-$MgF_3$-TSA complexes of (a) αGal1P ($F_A = -149.0$, $F_B = -153.5$, $F_C = -172.1$), (b) G6P ($F_A = -147.0$, $F_B = -151.8$, $F_C = -159.0$), and (c) 2deoxyG6P ($F_A = -143.5$, $F_B = -149.7$, $F_C = -177.1$) recorded in 50 mM $K^+$ HEPES buffer pH 7.2 in 100% $H_2O$ at 25 °C. Free $F^-$ resonates at −119.0 ppm.

present in the $^{15}$N-HSQC spectrum of the PGM-$MgF_3$-αGal1P-TSA complex, as reported previously for other PGM-$MgF_3$-sugar phosphate-TSA complexes.[2f]

The backbone amide proton and nitrogen chemical shifts obtained from resonance assignment of uniformly $^2$H,$^{13}$C,$^{15}$N-labeled β-PGM in the PGM-$MgF_3$-αGal1P-TSA complex were used to derive chemical shift changes ($\Delta\delta$) on switching from the PGM-$MgF_3$-G6P-TSA complex to the PGM-$MgF_3$-αGal1P-TSA complex (Supporting Information). Overall, the observed changes are small but widespread, while the lack of significant $\Delta\delta$ for the hinge regions between the cap and the core domains (T14-D15 and V92-Y93) shows that the two complexes adopt very similar closed conformations. By contrast, residues of both of these PGM-$MgF_3$-TSA complexes show significantly different crosspeak distributions and line-broadening behavior to those of the open β-PGM conformation.[2c] In combination, all the above data unambiguously identify the existence of a stable, closed PGM-$MgF_3$-αGal1P-TSA complex in solution where the $MgF_3^-$ moiety is coordinated axially by αGal1P and the D8 carboxylate group, and is hydrogen bonded to the enzyme as in the PGM-$MgF_3$-G6P-TSA complex.

The presence of fluorine nuclei in near-transition state conformations for phosphoryl transfer allows an interrogation of the electronics associated with the active site architecture, by means of $^{19}$F NMR spectroscopic parameters. $^{19}$F chemical shifts, which are very sensitive to the electron distributions in the vicinity of the fluorine nuclei, display a high degree of dispersion and can be predicted with good precision.[4] Moreover, proton distributions in close proximity to the fluorine atoms can also be established on the basis of hydrogen/deuterium primary isotope shifts (sum isotope shifts) of the $^{19}$F resonances.[2f] For the PGM-$MgF_3$-G6P-TSA complex, $F_A$ in the $MgF_3^-$ moiety (Figure 1) is coordinated by three protons (L9H$^N$, D10H$^N$, and S114H$^O$) in a distorted tetrahedral arrangement, whereas $F_B$ and $F_C$ have trigonal coordination involving two protons (A115H$^N$ and K145H$^{N\zeta}$) and one proton (G6P-2′H$^O$), respectively. The $^{19}$F chemical shifts and sum isotope shifts of resonances $F_A$, $F_B$, and $F_C$ of the PGM-$MgF_3$-αGal1P-TSA complex can be compared to data obtained for the PGM-$MgF_3$-G6P-TSA and the PGM-$MgF_3$-2deoxyG6P-TSA complexes (Figure 2 and Table S1). For resonances $F_A$ and $F_B$, chemical shifts and isotope shifts are broadly similar for the three TSA complexes indicating that there are no significant differences in proton and electron distributions at these fluorine nuclei. However for $F_C$, the data display similarities for the PGM-$MgF_3$-αGal1P-TSA and the PGM-$MgF_3$-2deoxyG6P-TSA complexes but are distinctly different from those of the PGM-$MgF_3$-G6P-TSA complex. In the PGM-$MgF_3$-2deoxyG6P-TSA complex, the absence of the C(2)-hydroxyl group, which would normally coordinate $F_C$, leaves this fluorine nucleus without a hydrogen bond donor. This results in an upfield shift for $F_C$ ($\Delta\delta = -18.1$ ppm) when compared to the corresponding value for the PGM-$MgF_3$-G6P-TSA complex and a fall in the isotope shift to close to zero (0.21 ppm). For the PGM-$MgF_3$-αGal1P-TSA complex the corresponding comparison for $F_C$ shows a similar upfield $\Delta\delta$ (−13.1 ppm) and low isotope shift (0.28 ppm), indicating that, again, a hydrogen bond partner is not present. Inspection of the structural overlay of the PGM-αGal1P complex with the PGM-$MgF_3$-G6P-TSA complex (Figure 1) reveals that both the αGal1P stereochemistry and relocation of the sugar phosphate ring away from D8 preclude any direct hydrogen bond between αGal1P and $F_C$, in agreement with the NMR data.

In conclusion, we have used a combination of $^1$H, $^2$H, $^{13}$C, $^{15}$N, and $^{19}$F NMR measurements to provide clear evidence for the existence of a stable, closed PGM-$MgF_3$-αGal1P-TSA complex and its formation in solution from its component species. The coordination of the $MgF_3^-$ moiety in this complex is very similar to that identified previously in the PGM-$MgF_3$-G6P-TSA complex. The small spectroscopic differences between the two complexes reflect the loss of a hydroxyl group in a position to coordinate the fluoride that bridges the catalytic and tbp magnesium ions, which results from αGal1P axially coordinating the tbp magnesium ion *via* the C(6)-hydroxyl group while G6P coordinates *via* the C(1)-β-hydroxyl group. This demonstration of a solution state PGM-$MgF_3$-αGal1P-TSA complex establishes the utility and importance of this approach to obtaining enzyme structures that are complementary to reactive intermediates in phosphoryl transfer catalysis and refutes the major remaining piece of evidence used to support the putative existence of a pentavalent phosphorane in complex with β-PGM.

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**Supporting Information Available:** NMR experimental methodology, analysis of β-PGM $\Delta\delta$ and $^{19}$F sum isotope shifts. The NMR chemical shifts for the PGM-$MgF_3$-αGal1P-TSA complex and the PGM-$MgF_3$-G6P-TSA complex have been deposited in the BioMagResBank, www.bmrb.wisc.edu (accession codes 16409 and 7234, respectively). This material is available free of charge via the Internet at http://pubs.acs.org.

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