Glycerol alters substrate binding in PTPLP:IP complexes

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Protein tyrosine phosphatase-like *myo*-inositol phosphatases (PTPLPs or phytases) follow an ordered, sequential dephosphorylation pathway that utilizes the abundant *myo*-inositol-1,2,3,4,5,6-hexa*kis*phosphate ($InsP_6$ or phytate; approximate charge of -6 to -9 at physiological pH) to produce lessphosphorylated *myo*-inositol phosphates (IPs) containing between one and five phosphoryl groups [1]. Ultimately, we aim to engineer PTPLPs by rational design to manipulate the substrate specificity and produce alternate IP products [2]. The engineered PTPLPs can then be used for large-scale production of IPs. To rationally design an enzyme, clear understanding of how the structure affects function is required. Therefore, the goal is to understand PTPLP substrate specificity at atomic resolution in order to identify and understand the structural determinants that govern substrate specificity. To this end, multiple crystal structures of different IP ligands in complex with IPases are necessary. This work focuses on two PTPLPs which have high activity towards $InsP_6$ and divergent hydrolysis pathways: Phytase A from *Mitsuokella multacida* (PhyAmm; a tandem repeat) and *Selenomonas ruminantium* (PhyAsr) [1, 3].

Glycerol is commonly used to protect proteins while stored frozen, for stabilizing and enhancing protein solubility, in cryocrystallography, and is also used during protein crystallization [4-5]. PhyAmm can be crystallized in the presence or absence of glycerol at concentrations suitable for cryoprotection. The presence of glycerol during crystallization produces larger crystals with increased stability than those grown in the absence of glycerol. However, when soaked or co-crystallized with the highly-charged IP substrates, the resulting structures have the IPs bound to the active site in a catalytically incompetent manner, with inorganic phosphate bound to the phosphate-binding loop (P-loop) and the IP bound above the inorganic phosphate. When the concentration of glycerol is decreased before the PhyAmm crystal is soaked with an IP the structure results in the IP bound in a catalytically competent manner. In the case of PhyAsr, glycerol is used strictly as a cryoprotectant. When PhyAsr is soaked with an IP solution which contains glycerol, the IP binds to PhyAsr in a manner inconsistent with the known pathway or in a catalytically incompetent manner. When PhyAsr is first soaked with the IP and then glycerol added as the cryosolvent, an alternate substrate binding mode is observed.

This work demonstrates that glycerol can have a significant effect on protein-substrate interactions when involving high-charge density substrates. In the case of PhyAmm, the substrate is incapable of binding the P-loop, while the PhyAsr substrate binds the P-loop with alternate binding modes.

1. A. A. Puhl, R. J. Gruninger, R. Greiner, T. W. Janzen, S. C. Mosimann and L. B. Selinger, *Protein Sci.*, **16**, (2007) 1368-1378.

2. X. G. Lei, J. D. Weaver, E. Mullaney, A. H. Ullah and M. J. Azain, *Annu. Rev. of Anim. Biosci.*, **1**, (2013) 283-309.

3. R. J. Gruninger, L. B. Selinger and S. C. Mosimann, *J. Mol. Biol.*, **392**, (2009) 75-86.

4. R. Sousa, *Acta. Cryst.*, 51, (1995), 271-277.

5. L. Vera, B. Czarny, D. Georgiadis, V. Dive, and E. A. Stura., *Cryst. Growth Des.*, **11**, (2011) 2755-2762.