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Role of the domain 4 rotation in PMM/PGM from P. aeruginosa during catalysis

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The enzyme phosphomannomutase/phosphoglucomutase (PMM/PGM) belongs to the alpha-D-phosphohexomutase enzyme superfamily. It catalyzes the reversible conversion of 1-phospho to 6-phospho sugars. The reaction mechanisms involves two phosphoryl transfers, with a 180° reorientation of the reaction intermediate during catalysis. Research performed by Regni et al. (2002) on the crystal structure of the protein shows that the enzyme has four domains arranged in a "heart" shape. Domains 1-3 share a similar tertiary fold and many interfaces with each other, while domain 4 is structurally unrelated and shares fewer interfaces to the other three domains. A comparison of the apo-protein and enzyme-substrate structures showed that there was a rotation of domain 4 upon ligand binding; the movement is located between residues 365 to 381 connecting domains 3 and 4. Based on the structural and evolutionary analysis we determined that the highly conserved residues(P368, S369, P369, Y17 and R262) all showed significant importance in the movement of the fourth domain upon ligand binding and were chosen for site-directed mutation. Alanine was chosen for all mutations in order to decrease the interactions between domain 4 and the rest of the enzyme. To see how the mutation effects the activity of the enzyme we will couple its reaction to that of glucose 6-phosphate dehydrogenase, pairing the formation of glucose 6-phosphate to NADH formation, and therefore monitoring the formation of NADH by its absorbance at 340 and comparing its activity to that of the wild-type. Catagorization of mutants P368 to Alanine and S369 to Alanine, have shown a decreased affinity of the enzyme to the substrate compared to that of the wild-type.