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## Role of the interdomain rotation of PMM/PGM from P. aeruginosa in catalysis


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The enzyme /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 mechanism involves two phosphoryl transfers, with a 180° reorientation of the reaction intermediate during catalysis. 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 interactions with each other, while domain 4 is structurally unrelated and has fewer interactions with 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 primarily localized between residues 365 to 381, which connect domains 3 and 4. Based on structural and evolutionary analysis we hypothesized that the highly conserved residues (P368, S369, P369, Y17 and R262) would be involved in the movement of the fourth domain upon ligand binding and these residues were selected for site-directed mutagenesis. These residues were mutated to alanine in order to change the interactions between domain 4 and the rest of the enzyme. To see how the mutation effects the activity of the enzyme, it will be assayed using a coupled reaction with glucose 6-phosphate dehydrogenase, that pairs the formation of glucose 6-phosphate to NADH formation, and can be monitored by measuring the formation of NADH by its absorbance at 340. By comparing the activity of mutants to wild-type enzyme, we see that the P368A and S369A mutants have a decreased affinity of the enzyme for substrate.