Crystal structures of cofactor-independent phosphoglycerate mutase and phosphoglycerate kinase delineate the role of domain movement in catalytic mechanism

Abstract

Glycolysis is the major energy conversion pathway in living organisms. To meet the primary energy demand of the cell, a group of eleven enzymes works in a concerted way to maintain the level of ATP in the cytosol even in the absence of molecular oxygen. Recent scientific advancements have established several correlations between pathogenicity and glycolysis. In this regard glycolytic enzymes have now been considered as the lucrative drug targets against several microbial infections. Enzymes those are involved in ATP production and apparently absent in human have the potential to be the prime drug targets. Phosphoglycerate kinase (PGK) and cofactor-independent phosphoglycerate mutase (iPGM) play a crucial role in the terms of energy conversion in glycolysis. Both the enzymes are bi-domain in nature and domain movement is critical for efficient catalysis. Between these two bi-domain enzymes PGK catalyzes the production of first two molecules of ATP and iPGM catalyzes the isomerization of 3-phosphoglycerate (3PG) to 2-phosphoglycerate (2PG) in a reversible manner. Lack of any substrate-bound structure of iPGM in open conformation is a serious impediment in dissecting the catalytic cycle involving substrate acquisition, entry to catalytic site, formation of product and finally product release. With an aim to elucidate the mechanism of domain movement as well as catalysis, PGK and iPGM from *S. aureus* have been cloned, over-expressed, purified and crystallized. The thesis describes the crystal structures of these two proteins along with substrate bound complexes in an attempt to delineate the mechanism of domain movement. This work reports the first crystal structure of substrate-bound iPGM complex in a partially open conformation that shows the substrate binding site and the catalytic site on two distinctly separate domains. A spring-loaded release mechanism has been established to clarify the domain movement correlating with the substrate binding, catalysis and the product release. From the detailed structural analysis of SaiPGM and binary complexes, a complete catalytic cycle has been established. The conformational dynamics of PGK has been studied by simulated dynamics and its potency as a future drug target has been probed by using a common antiviral drug. The interaction between PGK, iPGM and GAPDH has been determined by fluorescence spectroscopy resulting in a report of such interactions in prokaryotic system.