Biochemical and Structural Investigation of the Dynamic Regulation Mechanism of Pyruvate Kinase Muscle Isoform 2 using Amino Acids

Suparno Nandi and Mishtu Dey

Department of Chemistry, The University of Iowa, Iowa City, IA 52242, United States

suparno-nandi@uiowa.edu, mishtu-dey@uiowa.edu

Pyruvate kinase muscle isoform 2 (PKM2), a key enzyme in the glycolytic pathway and cancer cell proliferation process has been reported to be regulated by several hydrophilic and hydrophobic amino acids. We have tested the effect of asparagine, aspartic acid, and valine on PKM2. Enzyme activity assays and ligand binding studies show that hydrophilic amino acids asparagine and aspartic acid activate PKM2 by increasing its affinity to substrate phosphoenolpyruvate (PEP). On the other hand, hydrophobic amino acid valine inhibit the enzyme by reducing its substrate binding affinity. Fluorescence quenching studies indicate that the presence of PEP in the active site increases the enzymes affinity to asparagine/aspartic acid while decreasing the affinity to valine in the amino acid binding pocket. Gel filtration studies reveal that hydrophobic amino acids inhibit the enzyme by shifting the oligomeric state of PKM2 from tetramer to a mixture of tetramer:dimer/monomer. On the other hand, hydrophilic amino acids stabilize PKM2 in a tetrameric state. Crystal structure of PKM2 with asparagine, aspartic acid, and valine suggest that the side chain of hydrophilic amino acids play a part in PKM2 activation, while the main chain of hydrophobic amino acids is involved in inhibition of PKM2.