Structural basics of human muscle fructose-1,6-bisphosphatase activity

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Glucose is the main energy source in mammals where its homeostasis in blood is maintained by the balance of catabolic glycolysis on the one site and gluconeogenesis on the other hand. Fructose-1,6-bisphosphatase (FBPase) is an allosteric enzyme crucial for gluco- and glyconeogenesis pathways. It occurs almost in all living organisms. It catalyzes non-reversible hydrolysis of fructose-1,6-bisphosphate to fructose-6-phosphate and inorganic phosphate. Moreover, the enzyme plays a key role in the control of energy metabolism and glucose homeostasis. All FBPases require divalent cations**,** such as Mg^{2+}, Mn^{2+}or Zn^{2+}**,** for their activity to further enhance the catalysis. As one of the key enzymes vital for maintaining of glucose homeostasis, FBPase is permanently under the control of two reciprocally antagonistic hormones: insulin and glucagon. They both control the production and degradation of a competitive inhibitor of FBPase, fructose-2,6-bisphosphate (F-2,6-BP). Furthermore, the enzyme activity is regulated by two other inhibitors, AMP and Ca^{2+}.

Vertebrate genomes contain two distinct genes for two FBPase isozymes of liver and muscle. These proteins differ in the kinetics and immunological properties, as well as amino acid sequence. In addition muscle FBPase is about 100 times more susceptible to allosteric inhibitors and about 1000 times more sensitive to inhibition by Ca^{2+} than the liver isozyme. FBPases form a homotetramer of 222 symmetry with a upper and a lover dimer. The dimers can rotate with respect to each other, leading to the inactive T-state and active R-state conformations of FBPase. Despite of wealth biochemical and structural data accumulated mostly for liver FBPase in last half century, the molecular basis for a mechanism of their action is not clear.

The aim of my Ph.D. thesis was to investigate the structure of human muscle FBPase. In the cause of studies I solved eight crystal structures of the muscle enzyme in its various states. On the basis of the structures of inactive FBPase in T-state, I was able to describe and map the conformational changes that occur within the tetramer of FBPase. These changes occur due to the removal of the AMP inhibitor from protein molecules as a consequence of the additiv of magnesium ions. Furthermore, the fact that I was able to solve the structure of the active enzyme in the R-state gave me the unique possibility to understand the equilibrium state for the active enzyme in the tetrameric form. The upper and lower dimmers form ~~a~~ specific interactions which I called the "leucine lock". Its formation is possible by almost perpendicular orientation of the dimmers on relative to each other, following a subunits rotation during the activation of the enzyme. The hydrophobic “leucine lock” allows the creation of a hydrogen bond between the key residues, Asp187, which could not be created without such protection. In addition, using snapshots from three crystal structures of human muscle FBPase, I could demonstrate that the AMP-binding event is correlated with a β→α transition at the N-terminus of the protein and with the formation of a new helical structure.