The First Crystallographic Structure of Mammalian Phosphofructokinase from Rabbit Muscle Skeletal Muscle

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Phosphofructokinase (PFK, EC 2.7.1.11) is a major allosteric enzyme which catalyzes the ATP dependent phosphorylation of fructose 6-phosphate to fructose 1,6-bisphosphate, one of the rate limiting steps of the glycolytic pathway (1). The crystallographic structure of a mammalian PFK has been a quest for biochemists and enzymologists for more than four decades. However, due to various levels of heterogeneity in the "purified" PFK samples such as the presence of isozymes, partial phosphorylation, proteolysis and most importantly, conformational heterogeneity, crystals of mammalian PFK have not previously been analyzed by X-ray diffraction. The lack of the crystal structure of mammalian PFK presents the major obstacle to the understanding of the structural basis of the allosteric control of these enzymes and the role of this regulation in health-related problems. This work presents the first crystal structure of a mammalian PFK from rabbit skeletal muscle (RmPFK). Recombinant RmPFK mutated at a unique site was crystallized. X-ray diffraction data at 3.2 Å were collected at the DORIS synchrotron of the EMBL-Hamburg Outstation. The structure of RmPFK was solved by molecular replacement using the program PHASER and the alpha-subunit of yeast PFK (ScPFK) as a starting model. As predicted from its amino acid sequence (2), the structure of an 80 kD RmPFK subunit resembles a pair of adjacent subunits of bacterial PFK (3). A RmPFK subunit has three nucleotide binding sites, including two ATP sites in the N-half and a unique ADP binding site located at the interface between the N-half and the C-half, which could be the ADP activation site of RmPFK. By combining the crystallographic structure of ScPFK, a dimer of RmPFK and the results from mutagenic studies on the latter (4, 5), a tetrameric structure for RmPFK and a proposed mechanism of its allosteric control have been deduced.

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