

Yeast Phosphofructokinase: Modeling Four Bacterial Tetramers to Act in Concert.

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Glycolysis is essential for the energy metabolism of living cells. Thus, phosphofructokinase (Pfk); a key regulatory enzyme of the glycolytic pathway, is expressed ubiquitously in all cellular organisms. The reaction catalyzed by Pfk, the phosphorylation of fructose 6-phosphate (F6P) to fructose 1,6-bisphosphate in the presence of ATP, represents the first irreversible step in the glycolytic pathway under physiological conditions. In prokaryotes, Pfk is a tetramer composed of four identical subunits of 35kDa in molecular mass and it is only regulated by two effectors (ADP as an activator and phosphoenolpyruvate as an inhibitor). On the other hand, eukaryotic-Pfks are substantially larger enzymes that exhibit a much more complex regulatory mechanism with over 20 effectors regulating their activity [1]. A deficiency or malfunction of Pfk can lead to muscle and non-muscle related diseases including hemolytic anemia, Alzheimer's disease and diabetes. Thus, a thorough understanding of the structure/function relationship of Pfk is needed to gain more insights into the mechanism of these diseases.

A combination of X-ray crystallography and biochemical data has yielded an understanding of the structure of the bacterial enzyme and of the conformational changes occurring between the active and inactive states (reviewed in [2]). However, of the eukaryotic enzymes only the structure of the *Trypanosoma brucei* Pfk, which shares 20-30% homology with bacterial-Pfk, is available [3]. The structure shows a striking similarity to its bacterial counterpart which serves as structural core modified by small insertions (loops) and larger N- and C-terminal extensions. The Pfk subunits of higher eukaryotic- have evolved from the bacterial gene by gene duplication, mutagenesis and fusion events, resulting in an N-terminal catalytic region paired with a C-terminal regulatory region [4]. Although to date no crystal structure has been published, several medium resolution Pfk 3D structures have been determined by both cryo-electron microscopy and negatively stained electron microscopy for the three yeasts (*S. cerevisiae*, *S. pombe* and *P. pastoris*) in various conformational states [5-9].

Our study focuses on both *S. cerevisiae* and *S. pombe* Pfks which form octameric enzymes and are closer related to the mammalian Pfks than either the bacterial or *T. brucei* Pfk. The α and β subunits of *S. cerevisiae* and the α subunit of *S. pombe* (with mol. masses larger than 100 kDa) can be described as consisting of two bacterial subunits each fused together by a linker (~50aa) with additional large N-terminal (~200aa) and C-terminal (~80aa) extensions. Bacterial-Pfk fits well into yeast-Pfk electron density maps derived by electron microscopy [7]. Surprisingly, the bacterial tetramer core (representing an $\alpha\beta$ dimer of *S. cerevisiae*) remains intact, despite the big linker and the *S. cerevisiae* Pfk octamer could be thought of as a complex comprising of 4 bacterial tetramers. However, the four bacterial cores account for less than 2/3 of the total molecular mass of the yeast Pfk octamer and cannot explain neither the interactions between the yeast tetramers nor the underlying structure for the sophisticated regulatory mechanism of eukaryotic Pfks. In the absence of a "good" crystal structure, we have used a combination of sequence alignment, homology modeling, and flexible fitting guided by our electron density maps together with available biochemical data to build models for the yeast Pfks. These models provide an interesting starting-

point for understanding the concerted motions during the active to the inactive state transition.

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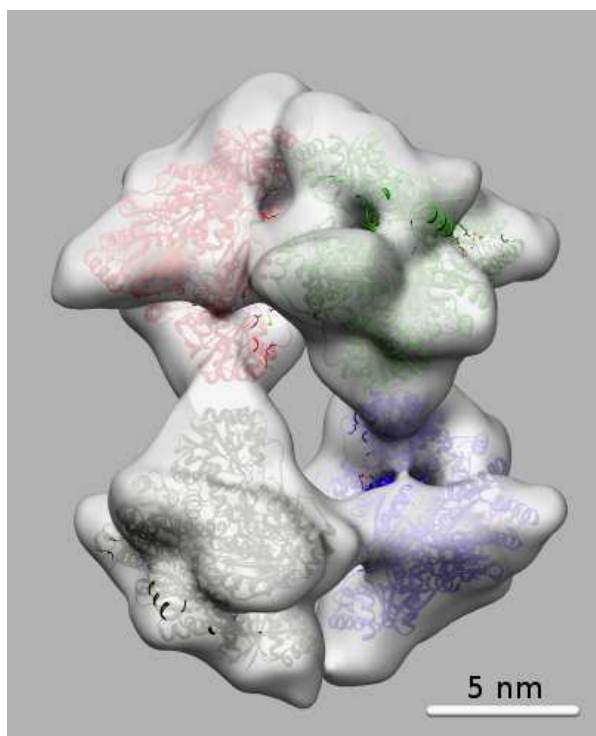


Figure 1: Cryo-electron microscopy map of the octameric *S. cerevisiae* Pfk in the active state with four bacterial tetramers fitted in as ribbons (red, green, black and blue).