The Structure of Yeast Phosphofructokinase 1

Wojciech Rypniewski[a], Katarzyna Banaszak[a], Ingrid Attinost[b], Gerhard Kopperschlaeger[c], [a]*Institute of Bioorganic Chemistry, Polish Academy of Science, Poland.* [b]*EMBL, Hamburg, Germany.* [c]*Institute of Biochemistry, Leipzig University, Germany.* E-mail: wojtekr@ibch.poznan.pl

Phosphofructokinase 1 (PFK) catalyses the ATP-dependent phosphorylation of fructose 6-phosphate (Fru-6P) to fructose 1,6-bisphosphate, one of the principal regulatory steps in glycolysis.

The structure of 12S PFK from *S.cerevisiae*, a product of limited proteolysis of the native enzyme (known as 21S), has been solved at 2.9 Å resolution in complex with Fru-6P. This is the first crystal structure of eukaryotic PFK and one of the largest protein crystal structures known to date in atomic detail (approx. 600 kDa). We have determined the topology of the enzyme, the active site and the binding site of fructose-2,6-bisphosphate (Fru-2,6-P_2), the allosteric effector specific to eukaryotes. Still unknown is the effector binding site for ATP. A detailed interpretation has been carried out of the electron density map. The refined atomic model contains over 5,000 amino acid residues and Fru-6P bound in the active site and in the Fru-2,6-P_2 effecttor site We have examined the consequesces on the enzyme structure and function of the two gene-duplication events that occured in the yeast gene compared to the prokatyotic gene.

Keywords: allostery, metabolism, regulation and reaction mechanisms of enzymes