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Structural basis of allosteric regulation of eukaryotic phosphofructokinases

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The enzyme 6-phosphofructokinase (Pfk) catalyses the formation of fructose 1,6-bisphosphate from fructose 6-phosphate and MgATP and contributes to the control of glycolysis in prokaryotic and eukaryotic cells. The catalytic activity is tightly regulated in a wide variety of organisms by diverse positive (e.g. fructose-2,6-bisphosphate, AMP) and negative (e.g. ATP, citrate) effectors.

Eukaryotic Pfk has evolved by a process of tandem gene duplication and fusion to yield a protein with a much more complex structural organization and allosteric regulation. The N-terminal half of a Pfk subunit obviously retained the catalytic function, whereas in the C-terminal half allosteric ligand binding sites have evolved from former catalytic and regulatory sites.

Pichia pastoris Pfk (PpPfk) is with ~1 MDa the most complex and probably largest eukaryotic Pfk [2]. We have determined the crystal structure of full-length *Pp*Pfk to 3.05 Å resolution in the T-state [1]. *Pp*Pfk forms an $(\alpha\beta\gamma)_4$ dodecamer of D_2 symmetry with dimensions of 161 x 157 x 233 Å mainly via interactions of the α-chains. The N-terminal domains of the α- and β-chains have folds that are distantly related to glyoxalase I, but the active sites are no longer functional. Interestingly, these domains located at the two distal ends of this protein along the long two-fold axis form a $(\alpha\beta)_2$ dimer as does the core Pfk domains, however, the domains are swapped across the tetramerization interface. In *Pp*Pfk, the unique γ-subunit participates in oligomerization of the αβ-chains. This modulator protein was acquired from an ancient SAM-dependent methyltransferase. The identification of novel ATP binding sites, which do not correspond to the bacterial catalytic or effector binding sites, point at marked structural and functional differences between bacterial and eukaryotic Pfk.

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Molecular mechanism of metal selective uptake by neutrophil-activating protein

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Helicobacter pylori is a pathogen associated with gastric diseases, including gastric ulcers and stomach cancers. *Helicobacter pylori* neutrophil-activating protein (HP-NAP) is reported to be a major 17 kDa antigen of *H. pylori*. This protein promotes the neutrophil adhesion to endothelial cells and the production of reactive oxygen species which is thought to be a cause of cancer. HP-NAP is a ferritin-like iron storage protein and forms a hollow spherical dodecamer whereas ferritin forms 24mer. Crystal structure of HP-NAP was solved in 2002 at 2.5 Å resolution[1]. The structure contained a Fe atom per subunit at a ferroxidase center. We previously determined the metal-free structure at 2.2 Å resolution[2]. The crystal shows higher symmetry (space group: *F*432, Zanotti's one: $P2_1$) and a high water content of 65.9% (*Vs*). Therefore, the *F*432 crystal is worthwhile for soaking experiments. In comparison with Zanotti's structure, the large conformational changes by the metal coordination were observed for three amino-acid residues (His37, Asp52 and Glu56). In order to estimate the selective mechanism of iron uptake, storage and elimination, we have determined the crystal structures containing various metal ions.

HP-NAP was expressed as a His-tag fused protein and crystallized using the hanging drop vapor diffusion method at 20 °C. To prevent the protein from aggregation, 0.1 M L-arginine was added during purification and crystallization processes. Crystals were grown against 25% ethylene glycol precipitant. By cocrystallization method, crystals including Fe^{2+} or Cd^{2+} ions were obtained using the reservoir solution containing each metal ion. Soaking methods were also performed for Fe^{2+} and Zn^{2+} ions. Anomalous difference data were collected at the Photon Factory in Japan. Ferrous ions incorporated into HP-NAP are probably oxidized to ferric ions instantly by HP-NAP's ferroxidase activity. When Fe^{2+} is added to the droplet, the crystal color is changed to be tinged with yellow. Therefore, Fe atoms in the structure were assigned as ferric ions and refined.

Cd^{2+} and Fe^{3+} structures through cocrystallization were obtained at 2.2 and 2.7 **Å** resolution, respectively. Each subunit contains fourteen cadmium ions and two ferric ions, respectively, and these ions were chelated in a different manner. Fe^{3+} and Zn^{2+} structures obtained through soaking method, were refined at 3.0 and 2.5 **Å** resolution, respectively. Three ferric ions and six Zn^{2+} ions were chelated in ordinary mononucleated manners. Number of metal ions through soaking method is greater than that through cocrystallization. Negative-charged residues are abundant in the inner surface of HP-NAP, which contributes to this additional iron storage mechanism. The most major metal ions were located at the ferroxidase center of the subunit interface. At these metal chelation sites, metal ions adopt the different coordination involved in one or two water ligands. Zn^{2+} and Cd^{2+} ions form binuclear coordination and Fe^{3+} builds mononucleated chelation.

There are two different kinds of pore channels, one of which must be the selective metal ion path from the outer hydrophilic shell to the inner space and vice versa, and are formed at the 3-fold rotational symmetry axes. In Fe^{3+} and Zn^{2+} structures, metal ions are clearly observed at one of the pores.

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Crystal structure of the allosteric-defective chaperonin $GroEL_{E434K}$ mutant

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