The enzyme 6-phosphofructokinase (Pfk) catalyzes 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.

The structure of the allosteric-defective chaperonin GroEL was determined by the University of Leipzig. The crystal was grown against 25% ethylene glycol precipitant. By co-crystallization method, crystals containing Fe2+ or Cd2+ ions were obtained using the reservoir solution containing each metal ion. Soaking methods were also performed for Fe2+ and Zn2+ 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 Fe2+ is added to the droplet, the crystal color is changed to tinged with yellow. Therefore, Fe atoms in the structure were assigned as ferric ions and refined.

Cd2+ and Fe2+ structures obtained through co-crystallization were observed 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 different manners. Metal ions obtained through soaking method, were refined at 3.0 and 2.5 Å resolution, respectively. Three ferric ions and six Zn2+ ions were chelated in ordinary mononucleated manners. Number of metal ions through soaking method is greater than that through co-crystallization. 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 most major metal ions were located at the ferroxidase center of the subunit interface. At these metal chelation sites, metal ions adopt the

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 Fe2+ and Zn2+ structures, metal ions are clearly observed at one of the pores.


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