s1.m8.p28 **Crystal Structure of Histidinol phosphate Phosphatase: Coordination of Three Metal Ions.** Omi, R.[1,3], Goto, M.[1,3], Miyahara, I. [1,2] and Hirotsu, K.[1,2], [1]*Graduate School of Science, Osaka City University, Japan,* [2]*Harima Institute/SPring8, The Institute of Physical and Chemical Research (RIKEN), Japan, 3Graduate School of Science, Osaka University, Japan. E-mail: omi@sci.osaka-cu.ac.jp*

Keywords: Phosphatase; Crystal structure; Metal ion

Histidinol phosphate phosphatase (HisPPase) catalyzes the eighth step of the histidine biosynthesis, in which L-histidinol phosphate undergoes dephosphorylation to give histidinol. The HisPPases are divided into *Escherichia coli* and *Bacillus substilis* type. One is the bifunctional enzyme in which the N-terminal domain has HisPPase activity and the C-terminal domain has imidazol-glycerol phosphate dehydratase activity. The enzyme belongs to the 'DDDD' superfamily named after the presence of the four invariant aspartate residues. The other is a monofunctional enzyme with HisPPase activity. The enzyme belongs to the 'PHP' superfamily named after polymerase and histinol phosphate phosphatase, the catalyric site of which has four motifs with conserved histidine residues.

Three-dimensional structures of HisPPase in its unliganded form and complexed with the phosphate analogue SO_4^{2-} have been determined at 1.75 and 1.7 Å resolution, respectively. HisPPase which is a monofunctional enzyme has been expressed in *E. coli*, purified and then crystallized by the hanging-drop vapor-diffusion technique. The crystal complexed with sulfate belongs to the orthorhombic space group $P2_12_12$ with unit-cell parameters a = 84.8, b = 97.2, c = 74.9 Å and the native crystal belongs to the orthorhombic C2221 with a = 77.0, b = 157.6, c = 117.0 Å. The crystals most likely contain two monomers in the asymmetric unit with V_M values of 2.57 $Å^3$ Da-1 for the complex crystal and 2.96 $Å^3$ Da^{-1} for the native crystal.

The crystal structure of HisPPase was solved by the multiple isomorphous replacement method using four isomorphous data sets. The refined HisPPase structure comprises two subunits in an asymmetric unit. The enzyme is a homo tetramer with a crystallographic 2-fold axis. The subunit of HisPPase has a pseudo barrel structure of seven β-strands and α-helices with an anti-parallel β-sheet of a C-terminal. The overall structures of the native enzyme and its complex with sulfate are essentially the same. The active site is formed at the top of the barrel. The difference Fourier map clearly exhibited three large peaks corresponding to metal ions at the active site. The XAFS analysis of HisPPase crystal indicated that Fe and Zn ions are bound to the active site of HisPPase. Using an anomalous Fourier map, two peaks were identified as metal binding sites for Fe(III) and Zn(II), but the remaining one peak (M3) has not yet been identified. The Zn(II) and Fe(III) are coordinated in a tetrahedral and an octahedral manner, respectively, while M3 is five-coordinated in a square pyramidal conformation. The histidine residues conserved in four motifs are coordinated to these three metal ions. It is assumed that a hydroxide ion which bridges Fe(III) and M3 plays an important role in catalytic action.

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s1.m8.p29 **Structure-Function Studies on Prolyl Oligopeptidase.** Dean Rea[a], Zoltan Szeltner[b], László Polgár[b] and Vilmos Fülöp[a], [a]*Department of Biological Sciences, University of Warwick, Coventry, UK.* [b]*Institute of Enzymology, Hungarian Academy of Sciences, Budapest, Hungary. E-mail: dean_rea@yahoo.co.uk*

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Prolyl oligopeptidase hydrolyzes the peptide bond on the C-terminal side of proline in peptides up to around 30 amino acid residues. The enzyme is involved in hormone and neuropeptide processing and is implicated in amnesia, depression and blood pressure regulation. Crystal structure determination of porcine prolyl oligopeptidase revealed a two domain structure in which the catalytic domain has an alpha-beta hydrolase topology similar to lipases and esterases [1]. The Ser-Asp-His catalytic triad is covered by the central tunnel of an unusual seven-bladed open-topology beta propeller domain which lacks a molecular 'velcro' between the first and last blades. Oscillation of the propeller blades serves as a gating filter that excludes large structured peptides, protecting cytosolic proteins from proteolysis [2]. The mechanisms of catalysis and regulation were further investigated using a combination of site-directed mutagenesis, kinetic measurements, X-ray crystallography and synthetic peptide chemistry. Enzyme variants containing engineered disulphide bridges demonstrated the requirement for concerted movements of the peptidase and propeller domains in addition to separation of the propeller blades during substrate entry. Substrate peptides displaying different kinetic profiles were shown to form similar enzyme-substrate complexes, highlighting the importance of enzyme-substrate interactions that occur on route to the Michaelis complex [3]. Variants lacking the catalytic aspartic acid revealed the catalytic importance of this residue. However, it is not required to stabilize the catalytically competent position or tautomer of the catalytic histidine [4]. A variant with a deficient oxyanion binding site demonstrated the importance of electrophilic catalysis for oxyanion stabilization [5]. The catalytic contributions of the catalytic aspartic acid and the oxyanion binding site are more important during hydrolysis of a peptide with a stronger scissile bond.

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