Crystal Structure of *Vibrio alginolyticus* Aminoacylhistidine Dipeptidase, a Carnosine-hydrolyzing Enzyme Relating to Biofilm Formation

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Aminoacylhistidine dipeptidase (PepD, EC 3.4.13.3) is a member of the metallopeptidase family M20 from the metallopeptidase H (MH) clan. It catalyzes the cleavage and release of an N-terminal amino acid, usually neutral or hydrophobic residue, from Xaa-His dipeptide or degraded peptide fragments for amino acid utilization. We have cloned, over-expressed, and purified the wild-type protein and characterized its biochemical properties including substrate specificity, pH and temperature optima, and effects of metal ions substitution. Mutational analysis of the putative active-site residues supported the involvement of His80, Asp119, Glu150, Asp173, and His461 as well as Glu149, Asn260, Arg369, and Gly435 in metal-binding and substrate recognition, respectively. Furthermore, crystallization and X-ray structure determination of the PepD protein revealed a "catalytic domain" and a "lid domain", which are similar to some kinds of other dipeptidases. The resolved crystal structure of the aminoacylhistidine dipeptidase provides in-depth insight for putative active site pocket validation. The results of this study may serve as a model for understanding the structure-activity relationships of carnosinase and metalloprotease superfamily in general and for future application of antibody-directed enzyme prodrug therapy for neuroprotection or cancer therapy.

Crystal structure of *V. alginolyticus* PepD

The *V. alginolyticus* PepD crystals were obtained by using hanging drop mothod. The crystals were observed at 20 ºC in 6 months by the best condition: crystallization reagent was composed of 28% PEG-400, 0.1 M Na-HEPES, 0.2 M $CaCl_2$, pH 7.5. Crystals of diamond-shaped grew in 6 months to maximum dimensions are about 0.3 x 0.2 x 0.1 mm (Fig. 1).

Fig. 1: A single crystal of *V. alginolyticus* PepD protein grown by hanging drop method.

Collecting X-ray data

Analysis of the diffraction pattern indicated that the crystals exhibit the hexagonal symmetry and the space group should be $P6_5$. Assuming two molecules per asymmetric unit, we estimated the Matthew's coefficient to be 2.63 $Å^3$/Da, corresponding to solvent content 53.4 %, which is within the normal range for protein crystals.

Initial attempts to solve the crystal structure of PepD from *Vibrio alginolyticus* were performed with the molecular replacement method using the structure of Xaa-His dipeptidase from *Haemophilus somnus 129PT* (PDB code: 2QYV) as a search model that shows sequence identities of 50.9%. A molecular replacement solution was found by using the *CCP4 (MOLREP)* program after the rotation and translation search using the resolution range 25-4.0 Å, and confirmed that the space group belongs to $P6_5$ and there are two molecules in an asymmetric unit (Fig. 2). The metal ions binding sites are showed in Fig. 3, and the distance between metal ions and ligands are showed in Table 1.

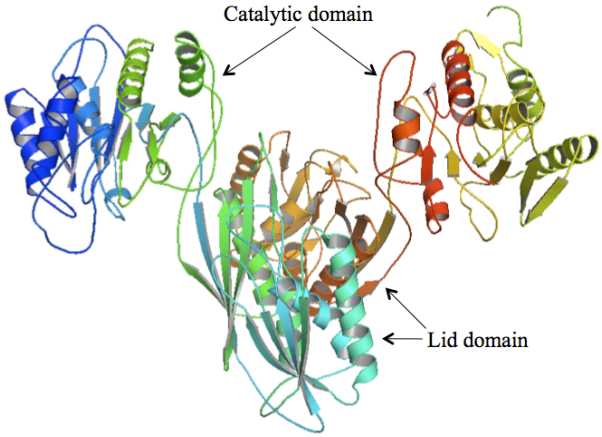

Fig. 2: Ribbon diagram of preliminary *V. alginolyticus* PepD structure It shows a homodimer, and each monomer has a catalytic domain and lid domain.

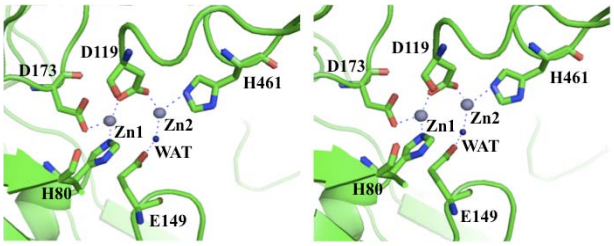

Fig. 3: Showing the two zinc ions and their ligands in ball and stick, respectively. The Zn1 is coordinated by bridging Asp119, H80 and D173. The Zn2 is coordinated by bridging Asp119, H461 and one water molecular (deep blue). The water molecular is bound by carboxylate oxygen of E149 through Hydrogen bond.

Table 1: The distance between zinc ions and ligands.

Ligand	Ligand-Zinc 1 (Å)	Ligand-Zinc 2 (Å)
Asp173 Oδ1	2.1	
Asp173 Oδ2	2.3	
His80 Nε2	2.4	
Asp119 Oδ1	2.2	
Asp119 Oδ2		2.0
His461 Nε2		2.3
Glu149 Oε2		3.2
WAT O		2.5