tures support a mechanism of phosphate ester hydrolysis involving interaction of the substrate with Zn(II) followed by a nucleophilic attack on the phosphorus by an Fe(III)-coordinated hydroxide ion. The negative charge evolving at the pentacoordinated transition state is probably stabilized by interactions with the divalent zinc and the imidazole groups of His-202, His-295 and His-296, the later protonating the leaving alcohol group.

[1] N. Sträter, T. Klabunde, P. Tucker, H. Witzel & B. Krebs, *Science* **268**,1489

PS04.02.30 CRYSTAL STRUCTURE OF A EUKARYOTIC (PEA SEEDLING) COPPER-CONTAINING AMINE OXIDASE AT 2.3Å RESOLUTION. Vinay Kumar, Hans C. Freeman and J. Mitchell Guss (University of Sydney, NSW 2006, Australia); David M. Dooley and Michele A. McGuirl (Montana State University, Bozeman, MT 50717, USA.)

We report the first structure analysis of a eukaryotic amine oxidase, pea seedling amine oxidase (PSAO), at 2.3Å resolution. The structure was solved using phases derived from a single heavy-atom (phosphotungstic acid, $H_3PW_{12}O_{40}$) derivative. The positions of the tungsten atoms in the W_{12} cluster were obtained by molecular replacement using the prokaryotic amine oxidase from *E. coli* (ECAO) [Parsons, M.R. *et al.* (1995). *Structure*, **3**, 1171-1184] as a search model. However, the methodology avoided bias from the search model and resulted in an essentially independent view of a eukaryotic amine oxidase molecule.

Copper-containing amine oxidases are a widely distributed class of enzyme whose function is to catalyze the oxidative deamination of biogenic amines to the corresponding aldehyde. The redox reaction is facilitated by an organic cofactor, topa quinone (TPQ), which is formed by the post-translational modification of an invariant Tyr residue.

The PSAO molecule is a homodimer with dimensions 100x63x42 $Å^3$. The copper(II) atom at the active site of each subunit is coordinated by three histidine side chains and two water molecules in an approximately square-pyramidal arrangement. All the atoms of the topa quinone (TPQ) cofactor are unambiguously defined. The closest contact to the copper atom is ~6Å. A second metal atom revealed by the structure analysis is tentatively identified as manganese(II).

The molecular structure of PSAO is similar to that of the prokaryotic ECAO. A detailed comparison of the two structures suggests that the TPQ side chain is sufficiently flexible to move between uncoordinated and coordinated positions with respect to the copper atom. Such flexibility may be associated with the different spatial requirements for TPQ biogenesis and amine oxidation.

PS04.02.31 CRYSTAL STRUCTURE OF A ZINC METALLOENDOPROTEASE FROM *STREPTOMYCES CAESPITOSUS* AT 1.6Å RESOLUTION. G.Kurisu, A.Sugimoto, Y.Kai and S.Harada†, Department of Applied Chemistry, Faculty of Engineering, Osaka University, Suita, Osaka 565, Japan, †Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan

A zinc protease from *Streptomyces caespitosus* (ScNP), which is specific for peptide bonds on the amino side of aromatic residues, consists of 132 amino acid residues with one disulfide bond. While ScNP has the zinc-binding sequence His83-Glu-Xaa-Xaa-His87, it does not share overall significant similarity to the sequences of other zinc proteases (S. Harada, T. Kinoshita, N. Kasai, S. Tsunasawa and F. Sakiyama, (1995). *Eur. J. Biochem.* **233**, 683-686).We crystallized ScNP and determined its three-dimensional structure at 1.6 Å resolution. The structure analysis was performed by the MIR method. The crystallographic R-factor of the structure refined by XPLOR and PROLSQ was 0.16. ScNP consists of a highly twisted five-stranded β-sheet, four α-helices, one catalytically essential zinc ion and one calcium ion as shown in Figure 1.

This structure is topologically similar to those of the catalytic domains of other zinc proteases such as atacin, thermolysin, serratia, snake venom and collagenase despite a lack of sequence homology. The zinc atom of ScNP is tetrahedrally ligated by His83 and His87 in the zinc-binding sequence, Asp93 and a water. ScNP is the first zinc endoproteases in which an aspartate ligates to the zinc, and thus represents a novel organization of zinc ligands.

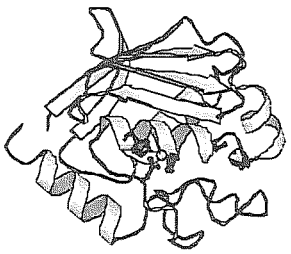

Figure. 1 Schematic drawing of ScNP[1)]

1)Kraulis, P.J. *J.appl.Crystallogr.* **24**, 964-950 (1991)

PS04.02.32 BACILLUS CEREUS NEUTRAL PROTEASE G197D AND E144S MUTANT STRUCTURES. S.A. Litster and P.W. Codding, Department of Chemistry and D.R. Wetmore, R.S. Roche, Department of Biological Sciences, University of Calgary, Calgary, Alberta, Canada, T2N 1N4

To gain an understanding of the role of calcium binding in the thermal stability of thermolysin-like neutral proteases, mutants of Bacillus cereus neutral protease (CNP) were developed and the structures determined. Here we present the crystal structures of the G197D and E144S mutants of CNP, at 3.0Å and 2.8Å resolution, respectively. A comparison of the structures and how they relate to the thermal stability and hydrolyase activity of the enzyme will be made. The G197D structure is novel in that it contains only three calcium ions, with the missing calcium ion being Ca(II); the ion thought to bind cooperatively along with Ca(I) to form the double calcium binding site in native CNP and thermolysin. The second structure, that of the inactive E144S mutant, the Glu to Ser mutation reduces the protease activity of the enzyme to 0.16% that of wild type and represents the first crystal structure of an active site mutant of a neutral protease. The mutant structure reveals a modified environment around the catalytic zinc ion and suggests a major role for bound water molecules. The mutants crystallize in the hexagonal space group P6(sub5)22 which is isomorphous with wild type crystals.

CNP: Pauptit R.A., Karlsson R., Picot D., Jenkins J.A., Niklaus-Reimer A., Jansonius, N., *J. Mol. Biol.* (1988), 199, 525-537.