Identification of Ca\textsuperscript{2+} ions bound to moderately large proteins by an X-ray anomalous dispersion technique seems to be possible using a high-energy synchrotron source and a sensitive imaging plate. With three kinds of proteins, Serratia protease from Serratia sp E-15 (Hantada, K. et al., 1995, J. Biochem., in press), alkaline protease from Pseudomonas aeruginosa IPO3080 (Miyatake, H. et al., 1995, J. Biochem. 118, 474) and Arthrobacter ramosus peroxidase (Kunishima, N. et al., 1995, J. Mol. Biol. 235, 331), the identification of Ca\textsuperscript{2+} ions in their crystals were carried out by the anomalous technique. The X-ray wavelengths used for data collection were 1.283 Å (synchrotron radiation) for Serratia protease, 1.488 Å (synchrotron radiation) for Arthrobacter ramosus peroxidase and 1.542 Å (Cu-K\textsubscript{α}) for alkaline protease. The determination of Ca\textsuperscript{2+} binding sites was performed by Bijvoet difference Fourier maps calculated with coefficients of (F\textsubscript{I} + F\textsubscript{II}) exp(iφ\textsubscript{α} - φ\textsubscript{β}/2) in the Serratia protease, each of five Ca\textsuperscript{2+} ions was located between two neighbors of loops in the β-sheet coil formed by the repeated sequence of GGXXDXBX (B: bulky hydrophobic residue, ideally leucine). In the alkaline protease whose tertiary structure is quite similar to that of Serratia protease, Ca\textsuperscript{2+} ions were identified at seven sites, six of which were shared with the Serratia protease. These Ca\textsuperscript{2+} ions in the both proteins are significantly important in stabilizing the β-sheet coil structure. In the peroxidase, the two Ca\textsuperscript{2+} ions were identified at the expected positions. The Ca\textsuperscript{2+} ions contribute to stabilization of the tertiary structure of the peroxidase.

NiFe hydrogenase from sulfate-reducing bacterium, Desulfovibrio vulgaris Miyazaki F is a membrane protein composed of heterodimer of 62.5 and 28.8 kDa. It has two Fe\textsubscript{3}S\textsubscript{4} clusters, and one nickel atom as active centers. The single crystals of hydrogenase was prepared from 40% of 2-methyl-2,4-pentanediol solution (space group is P 2\textsubscript{1} 2\textsubscript{1} 2\textsubscript{1}, a =101.0 b =126.5 c =66.5 Å). The structure determination has been carried out by the multiple isomorphous replacement (MIR) method combined with the multiwavelength anomalous diffraction (MAD) effect from native crystal. Six derivatives were successfully prepared and showed effective changes in their diffraction intensity. Native anomalous diffraction data were collected at five wavelengths (1.040, 1.489, 1.730, 1.743 and 1.750 Å) from one crystal. Native phases were obtained from MIR and MAD data set using the MLPHARE in CCP4 program package. The initial electron density map at 3.0 Å was not in good quality but had many ghost peaks around heavy atom binding sites. The quality of electron density map was slightly improved by iterative solvent flattening and histogram mapping. The main chain tracing was carried out at 3.5 Å map, and new molecular envelope was recalculated from this main chain backbone model. The phases were further improved by solvent flattening using this envelope. The new electron density map calculated at 3.0 Å was clear enough to locate the side chain atoms and even carbonyl oxygen atoms. The structure of hydrogenase is now in under refinement using the program XPLOR. The R-value was reduced to 0.30 from the initial model (0.510) by only positional refinement. The structure comparison with NiFe hydrogenase from the different strain, Desulfovibrio gigas will be discussed.
tutes 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.


PS04.02.20 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 59717, USA.)

We report the first structure analysis of an 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$_3$PW$_{12}$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, tosquinone (TPQ), which is formed by the post-translational modification of an invariant Tyr residue.

The PSAO molecule is a homodimer with dimensions 100x65x42 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 tosquinone (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.

A zinc protease from Streptomyces caesipitosus (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. Tsunawawa 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, serratin, 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


PS04.02.32 BACILLUS CEREUS NEUTRAL PROTEASE G197D AND E144S MUTANT STRUCTURES, S.A. Lister 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 hydrolyse 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$_3$ (sub5) 22 which is isomorphous with wild type crystals.