with the oil-water interface of a micellar substrate. PML activity is dependent on one catalytically important Ca^{2+} ion, presumably bound to its N-domain. However, the mechanism of this Ca^{2+} ion in conferring activity to PML was unknown. To answer the above questions, the crystal structure of PML was required. Recently we have solved the PML crystal structure to 1.48 Å resolution by SIRAS method using a Pt-derivatized crystal of S445C mutant, obtained by Cys-scanning mutagenesis. The structure showed that PML consists of an N-catalytic domain and a C-domain that contains two β-roll structures, stacked together in an antiparallel manner. The possible mechanism of the chaperone-like function of the β-roll structure will be discussed. Comparison of the closed and open structures of PML and the homologous *Serratia marcescens* lipase (SML) revealed the presence of two lid structures, the second one is novel to lipases. One buried Ca^{2+} ion is present in one lid structure in the open conformation (SML) and is absent in the closed conformation (PML). This Ca^{2+} ion functions to stabilize the open conformation against Coulombic repulsions.

Keywords: crystallography of biological macromolecules, enzyme structure, enzyme activity mechanism

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Hypervalent intermediate of archaeal peroxiredoxin

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Hypervalent intermediate of archaeal peroxiredoxin | Peroxiredoxins (Prxs) are thiol dependent peroxidases that reduce hydrogen peroxide and alkyl peroxides to water and the corresponding alcohols, respectively. In addition to antioxidant functions, Prxs maintain the intracellular level of hydrogen peroxide that affects signal mediators through its self-inactivation mechanism. The reaction of Prx is dependent on the redox active cysteine side chains. In general, the oxidation of a cysteine side chain of protein is initiated by the formation of cysteine sulfenic acid (Cys-SOH). Here, we demonstrate a novel mechanism of thiol oxidation through a hypervalent sulfur intermediate by presenting crystallographic evidence from an archaeal Prx, the thioredoxin peroxidase from Aeropyrum pernix K1. Oxidation by hydrogen peroxide converted the active site peroxidatic Cys50 of the archaeal Prx to a cysteine sulfenic acid derivative, followed by further oxidation to cysteine sulfinic and sulfonic acids. The crystal structure of the cysteine sulfenic acid derivative was refined to 1.77 Å resolution with R_{cryst} and R_{free} values of 18.8% and 22.0%, respectively. The refined structure, together with quantum chemical calculations, revealed that the sulfenic acid derivative is a type of sulfurane, a hypervalent sulfur compound, and that the S^{γ} atom is covalently linked to the N^{δ} atom of the neighboring His42. The reaction mechanism is revealed by the hydrogen bond network around the peroxidatic cysteine, as well as by the motion of the flexible loop covering the active site, and quantum chemical calculations. This study provides the first evidence that a hypervalent sulfur compound occupies an important position in biochemical processes.
Reference: Nakamura et al. (2008) PNAS, in press

Keywords: peroxiredoxin, peroxidatic cysteine, hypervalent compound

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Crystal structures of alkaline protease from *Pseudomonas aeruginosa* complexed with peptides

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Alkaline protease (AP, 467 amino acid residues) is a zinc endoprotease produced by *Pseudomonas aeruginosa* and most active at slightly alkaline pH with broad substrate specificity. The enzyme contains the consensus amino acid sequence (HEXXHXXGXXH) that is found in one class of zinc endoprotease called 'metzincins', in which the catalytically essential zinc ion is coordinated by the histidine residues of the sequence. In this study, we prepared crystals of AP complexed with a substrate and determined its structure as the first step to demonstrate the intermediate structure of the enzymatic reaction of AP. In order to prepare crystals of AP complexed with substrates, several peptides were synthesized and soaked into AP crystals of different crystal forms ($P2_12_12_1$, $P2_1$, and $P6_5$) at acidic pH, where the enzymatic activity was extremely low. X-ray structure analyses showed that crystals of the AP-substrate complex were obtained when the peptide, Arg-Pro-Lys-Pro-Gln-Gln (substance $P_{1\text{-}6}$), was soaked into $P2_12_12_1$ crystals. In this structure, the substrate carbonyl oxygen between Pro4 and Gln5 is ligated to the zinc ion located in the active center, which is the suitable binding mode for the hydrolysis of the peptide bond between Pro4 and Gln5. However, Arg-Pro-Lys-Pro-Gln-Gln-Phe (substance $P_{1\text{-}7}$) was bound to AP in two different modes in crystals. One is the same manner as substance $P_{1\text{-}6}$, but the carbonyl oxygen between Gln6 and Phe7 is ligated to the zinc ion in the other mode. Based on the determined structures, the enzymatic reaction mechanism of AP will be discussed. Currently, we are trying to prepare crystals of AP complexed with a reaction intermediate.

Keywords: zinc peptidase, X-ray structure analysis, substrate binding

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Crystal structure of YlqF, a circularly permuted GTPase

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The YlqF GTPase subfamily members are broadly conserved in eukaryotes, archaea, and bacteria, and include the stem cell regulator nucleostemin. In Bacillus subtilis, YlqF participates in the late step of 50S ribosomal subunit assembly and is targeted to a premature 50S subunit lacking L16 and L27 to assemble a functional 50S subunit through a GTPase activity-dependent conformational change of 23S rRNA. The GTPase activity of B. subtilis YlqF is stimulated by binding of the premature 50 S subunit. To provide the basis for understanding the biochemical functions of YlqF family GTPases,