in the molecule. This probably contributes to the activity expression of the present psychrophilic enzyme at low temperatures.

Keywords: biological macromolecules, dehydrogenases, cold adapted enzymes

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Crystallographic analysis of complexes of bovine trypsin and Schiff base metal chelate

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Studies on trypsin-specific compound are useful for the design of clinical useful compounds, since a variety of physiologically important enzymes (e.g. thrombin, kallikrein and urokinase) have trypsin-like specificity. Several benzamidine and phenylguanidine derivative have been reported to be potent inhibitor. In our previous papers, we have reported of the Schiff base copper(II) chelates carrying a guanidinium group. The chelates are strong inhibitor for trypsin (K_i =～10^{-5} M). To elucidate the structure-activity relationship in this novel series of inhibitors, the crystal structure of complexes between trypsin and guanidine-containing inhibitors were determined. The crystals (**1**,**2**) were obtained by equilibrating the droplet containing 1.25 mM trypsin, 0.1 M Tris-HCl buffer (pH 8.0), 22%(v/v) PEG 4000 and 0.2 M lithium sulfate. Guanidinium group of crystal **1** forms hydrogen bonds with Asp189O$^{\delta 2}$, Ser190O$^{\gamma}$, and Gly219O. The copper(II) ion of inhibitor is in close contact with His57 and Ser195. The imidazole nitrogen of His57 is directly coordinated with the copper(II) ion (2.33 Å). The copper(II) ion coordinated by the imine nitrogen, the phenolic oxygen and one carboxyl oxygen of the Schiff base ligand. In conjunction with detailed structural analysis, this approach will hopefully lead to the development of more potent inhibitor specifically targeting trypsin-like protease, as well as other physiologically important enzymes.

Keywords: X-ray crystallography of proteins, enzyme inhibitor design, medicinal chemistry

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Evolution of nylon-oligomer-degrading enzyme based on high resolution crystal structure analysis

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Carboxylesterase with b-lactamase folds on plasmid pOAD2 in *Arthrobacter* sp. KI72, was evolved to be a nylon-oligomer degrading enzyme through directed-evolution using error-prone PCR and DNA shuffling. The activity in a mutant (Hyb-S4M94) was enhanced 80-folds by substitutions of seven amino acid. Site-directed mutagenesis for parental carboxylesterase revealed that the enhancement of the activity is due to three mutations (R187S/F264C/D370Y). To understand the directed evolution of this enzyme based on the ultra-high resolution crystal structures, we have tried to grow the crystals in a good quality under the microgravity condition. X-ray crystal structure analyses of the various mutants and of S112A-activity-deficient-enzyme/Ald complexes were successfully carried out at high resolutions. In G181D mutant (20-folds activity) and in G181D/H266N double mutant (180-folds activity), substrate was stabilized in the active site by electrostatic interaction between amino group in Ald and D181-carboxyl. In contrast, in D370Y mutant (8-folds activity) and Hyb-S4M94, Ald was stabilized by hydrogen bonding and hydrophobic interaction between carboxyl group in Ald and Tyr370, located opposite side in the active site. In G181D/H266N/D370Y triple mutant, the kcat/Km value was increased 5-folds of the G181D/H266N double mutant, and the substrate was stabilized at both amino and carboxyl groups of Ald. Here we discuss the cumulative effects of amino acid substitutions for generating the catalytic centers responsible for nylon oligomer hydrolysis.

Keywords: nylon-oligomer-degrading enzyme, evolution of enzyme, high-reolution crystal structure analysis

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Structural biology study in biosynthesis of plant natural products

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Plants may be regarded as biofactories and synthesize over 200,000 natural products. Many of plant natural products can be used for the benefit of human and animal health. The biosynthesis of plant natural products is a very complex process including many different chemical reactions. We are working on three types of enzymes important for plant natural product biosynthesis, glycosyltransferases involved in glycosylation reactions, reductases involved in reductions, and cytochrome P450s involved in hydroxylation and dehydration. We determined crystal structures of several uridine diphosphate glycosyltransferases, NADPH-dependent reductases, and a cytochrome P450 enzyme. Theses structures provide essential insights into their structure-function relationships and catalytic mechanisms in the complex biosynthetic processes. Structure-based mutagenesis and the further functional study help to explore the roles of key residues for catalysis and specificity, and decipher the mechanisms. These studies may also provide us the basis for in vitro manipulation of enzyme activity and substrate specificity, and further rationally-based metabolic engineering and manipulation.

Keywords: enzyme structure function, crystal structures, biosynthesis