In plants, fungi and bacteria, aromatic amino acids are synthesized via the shikimate pathway. The pathway consists of seven enzyme-catalyzed reactions ending with the production of chorismate, a precursor of the amino acids phenylalanine, tryptophan and tyrosine, as well as a number of other aromatic compounds. The fourth step of the pathway, in which dehydroshikimate is reduced to shikimate, is catalyzed by shikimate dehydrogenase (SDH). In bacteria, kinetic and phylogenetic analyses have identified five SDH functional classes, annotated AroE, YdiB, RifI, SdhL and most recently, Ael1. Representative crystal structures have been determined for all classes except RifI. We are the first to present the crystal structure of the novel Ael1 homolog from *Pseudomonas putida*. SDH structures share a high degree of similarity and conservation of key residues involved in catalysis.[1] However, each SDH class has a distinct biochemical profile. While AroE is the archetypal SDH, YdiB has dual substrate specificity, accepting both shikimate and quinate.[2] RifI is predicted to accept amino-dehydroshikimate during the biosynthesis of the antibiotic rifamycin B.[3] The biological substrate of SdhL is unknown. Ael1 can catalyze the reduction of dehydroshikimate with a higher binding affinity but lower turnover rate than the AroE homolog. The natural enzymatic diversity observed among SDH classes provides an ideal system for studying modes of substrate selectivity.

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(2) Michel *et al*. (2003). *J. Biol. Chem., 278*: 19463-19472.
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Keywords: dehydrogenases, cocrystals, structure-function relationships

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Structure-function analysis of Eyes absent protein, aspartate dependent protein tyrosine phosphatase

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Protein tyrosine phosphatases (PTP)represent a large family of proteins involved in fundamental cellular signaling pathways. Eyes Absent(Eya) proteins have the peculiar characteristics that they do not contain the classical active site cysteine present in case of classical PTPs. Taking into account that the activity of this enzyme is depending on Mg^{2+} ions, determination of the 3D-structure would consistently help in evidencing the active site nucleophile and the general acid as well as the role played by the essential Mg^{2+} ion. We cloned full length human Eya3 gene from a human cDNA library and inserted it into pHAT2 prokaryotic expression vector. Recombinant protein thus obtained was further purified yielding pure enzymatic preparations. To evidence that the pure protein displays phosphatase activity several typical substrates were tested. pNPP and DiFMUP were found as efficient enzymatic substrates of hEya3. Using highly pure protein preparations various crystallization setups have been performed. Optimization of the promising precipitants led to needle-like microcrystals of hEya3. Further optimization of crystallization conditions as well as application of seeding procedures is expected to produce suitable crystals for diffraction experiments. Human Eya3 a non-typical protein tyrosine phosphatase was cloned from a cDNA library, the recombinant protein was expressed in prokaryotic system, purified to high purity and enzymatic activity was evidenced using typical phosphatase substrates. Optimization of crystallization setups produced microcrystals. Further increase of the microcrystal dimensions using additional crystallographic techniques expectedly will provide well diffracting crystals and finally the 3D- structure of new type of PTP.

Keywords: eyes absent, aspartate PTP, microcrystal

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First crystallographic structure of mammalian phosphofructokinase from rabbit skeletal muscle

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Phosphofructokinase (Pfk) is a key enzyme of the glycolytic pathway, which is present in all organisms. Pfk is the main regulatory point of the pathway and catalyses phosphorylation of fructose-6-phosphate to fructose-1,6-bisphosphate in the presence of ATP. Its molecular characteristic as well as its allosteric regulation by various effectors depend on the source of the enzyme. In bacteria the enzyme is a homotetramer with subunits molecular weight of about 35kDa, while most of the eukaryotic Pfks consist of “double-size” subunits when compared with the bacterial ones. Mammalian Pfk consists of subunits of about 80-85kDa molecular weight and is active as a tetramer or in more aggregated forms. The crystal structure of Pfk from rabbit muscle was determined to 3.2Å resolution. The protein model has two subunits in the asymmetric unit consisting of 748 amino acid residues. Nucleotides and phosphate ions have been found interacting with the protein molecules. The crystallographic model of Pfk from rabbit muscle is the first model of a eukaryotic Pfk which evolved by duplication and fusion of the genes as well as the first structure of Pfk from higher organisms. Amino acid sequence of the rabbit muscle enzyme shows 96% identity with the sequence of human muscle Pfk, so the knowledge of the rabbit muscle Pfk structure can be important for research on human Pfk and diseases related to this enzyme (Tarui disease).

Keywords: allosteric enzymes, metabolism enzyme, muscle proteins

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Crystal structure of a family I.3 lipase from *Pseudomonas* sp. MIS38 in a closed conformation

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Pseudomonas sp. MIS38 lipase (PML) is a family I.3 lipase, which is secreted by the type I secretion system. PML consists of two domains, an N-catalytic domain and a C-domain that contains a secretion signal and thirteen repeats of RTX (Repeats in ToXin) sequence motif that form β-roll structure(s) in the presence of Ca^{2+} ions. The β-roll structure was proposed to have a chaperone-like function with an unknown mechanism. PML has also been shown to exhibit interfacial activation, which indicate the presence of a lid structure that covers the active site and opens up upon contact

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,