reductase. We determined the X-ray structure of the key enzyme BoxB from *Azoarcus evansii* including the diiron center without and with bound benzoyl-CoA in the diferric and semi-reduced states, respectively [3]. Complementary Mössbauer studies in combination with the crystallographic data suggest that the semi-reduced state with bound benzoyl-CoA is a prerequisite for O_2 activation. The crystal structures reveal redox dependent structural changes, most significantly the movement of Glu150 from a diiron bridging in the oxidized, to a not ligating position in the semi reduced substrate bound state. In contrast to other members of the class I diiron enzyme family the position of benzoyl-CoA inside a 20 Å long channel is accurately known, indicating that the C2 and C3 atoms of its phenyl ring are closer to one of the irons (Fe1), and that the attacking oxygen of activated O_2 is essentially ligated to Fe1. We postulate a reaction cycle with an attack of an oxygen radical on C2. The unpaired electron is delocalized over the benzyl ring and the CoA thioester. The substrate bound structure indicates the stereoselective 2S,3R-epoxide formation by BoxB.

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keywords: diiron enzyme, mechanism, epoxidation

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Crystal structure of a zinc-dependent d-serine dehydratase from chicken kidney

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D-Serine is a physiological co-agonist of the N-methyl-D-aspartate receptor. It regulates excitatory neurotransmission, which is important for higher brain functions in vertebrates. In mammalian brains, D-amino acid oxidase degrades D-serine. However, we found recently that in chicken brains the oxidase is not expressed and instead a D-serine dehydratase degrades D-serine. The primary structure of the chicken DSD (chDSD) shows significant similarities to those of metal-activated D-threonine aldolases, which are fold-type III pyridoxal-5'-phosphate (PLP)-dependent enzymes, suggesting that it is a novel class of DSDs. chDSD catalyzes the dehydration of D-serine to form pyruvate and ammonia. In the catalytic reaction, a PLP-D-serine Schiff base (external aldimine intermediate) is formed at the first step. Our biochemical analysis suggested that in the following steps only the dehydration occurs despite the fact that the PLP-D-serine Schiff base is theoretically prone to five possible reactions.

In order to analyze the catalytic reaction mechanism of chDSD, we have determined the crystal structure of the native enzyme at 1.9 Å resolution. chDSD is a dimeric protein and has two active sites at the dimer interface. Each active site contains a PLP molecule, which forms a Schiff base with Lys45, and a zinc ion that coordinates His347 and Cys349. In order to elucidate the catalytic role of the zinc ion, we also determined the crystal structures of EDTA-treated chDSD, which lacks zinc ions in the active sites, and its D-serine complex. The crystal structure of EDTA-treated chDSD showed no significant conformational changes in the active site. An Fo(D-Ser)-Fo(Free) difference Fourier map of the EDTA-treated chDSD revealed that D-serine forms a Schiff base with PLP in the active site, suggesting that the zinc ion is not necessary to form an external aldimine intermediate. Since our biochemical analysis showed that addition of ZnCl2 recovers the enzyme activity of the EDTA-treated chDSD, the zinc ion seems to play a catalytic role after the formation of the external aldimine intermediate. The crystal structure of the EDTA-treated chDSD–D-serine complex showed that the binding mode of D-serine is not suitable for the Cα-H bond cleavage. According to Dunathan's hypothesis, the preferentially broken bond to Cα of the substrate should be nearly perpendicular to the Schiff base/pyridine ring plane. The Cα-H bond is in fact nearly parallel to this plane. A model building study of the chDSD–D-serine complex suggested that the interaction between the hydroxyl group of D-serine and the zinc ion is required to align the Cα-H bond perpendicular to the plane. In addition, when the substrate is properly aligned in the active site, the ε-NH2 group of Lys45 is located about 3 Å distance from the Cα atom of the substrate. Lys45 therefore seems to eliminate the α-proton from the bound D-serine. Our structural and biochemical studies suggested that the zinc ion is required to properly align the substrate in the active site and to assist the α-proton elimination. This is the first example of a PLP-containing enzyme that has a metal ion as a catalytic co-factor.

Keywords: dehydratase, D-serine, pyridoxal 5'-phosphate

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Molecular determinants of substrate specificity in a novel SGNH hydrolase

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AnAEst, a serine hydrolase from cyanobacteria *Anabaena sp*. PCC7120 is a member of the poorly studied SGNH hydrolase superfamily. Although the SGNH superfamily shows low overall sequence conservation, available structures display high structural homology with a conserved core α/β flavodoxin-like fold. These hydrolases display a diverse range of hydrolytic functions that include lipase, protease, esterase, thioesterase, arylesterase, lysophospholipase, carbohydrate esterase and acyltransferase activities, among others. Furthermore, they display broad substrate specificity, regio-specificity and enantio-specificity [1]. SGNH hydrolases possess a catalytic triad of absolutely conserved Ser and His residues and a mostly conserved Asp. The other conserved residues include a Gly and an Asn that are part of the active site and serve as the proton donors to the oxyanion hole.

Although the crystal structures of AnAEst in its apo-form and with an unknown intermediate of the catalytic Ser (PDB codes 1vjg, 1z8h) have been determined by the Joint Center for Structural Genomics, the biochemical properties and function of this enzyme are unknown. In this study, we report the biochemical characterization of AnAEst and provide a structural basis for activity and substrate specificity [2]. AnAEst is a homo dimer in solution and displayed arylesterase and thioesterase activities with high specificity for aryl esters of short chain carboxylic acids. AnAEst was regio-selective for α-naphthyl esters, with maximum activity at pH 7.5 and has a broad optimal temperature range (25 - 45 ºC). A structure based comparison of AnAEst with other superfamily members confirmed the presence of the catalytic triad and oxyanion hole (Ser17-Arg54-Asn87) residues. Interestingly, AnAEst exhibits a previously undescribed variation in the active site wherein the conserved Gly, a proton donor making up the oxyanion hole in this superfamily, is substituted by Arg54. Furthermore, AnAEst contained other unique variations in the active site, including a Leu instead of a conserved Gly and a Glu that makes a salt bridge interaction with Arg54. In order to better understand the molecular determinants of substrate specificity, the kinetic parameters of the wild-type and several

mutants of active site residues of AnAEst were determined. While the wild-type enzyme showed highest catalytic efficiency for naphthyl esters relative to phenyl esters, the R54G mutant displayed a 2.4 fold increase in catalytic efficiency for phenyl esters over naphthyl esters. The kinetic studies in conjunction with docking studies confirm the structural role of Arg54 and other active site residues in both substrate binding and catalysis.

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Structural insight into iron pathways in ferritin

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Ferritin directs the reversible biomineralization of iron. Crystals of apoferritin loaded, in aerobic conditions, with different amounts of $FeSO_4$ and $CuSO_4$ were studied by X-ray crystallography and the structure of the tripositive iron and bipositive copper adducts were determined at 2.7 Å and 2.8 Å resolution, respectively.

Anomalous diffraction experiments reveal the binding of the iron substrate to the ferroxidase site and to other sites in the protein, including a possible nucleation site for the iron mineral.

The metal coordination sphere at the catalytic site is redox dependent. The differences between iron and copper binding provide clues on the reaction mechanism and on the path of iron from the so-called C3 pore (entrance) to the C4 pore (exit into the cavity) through the catalytic site.

The crystallographic data combined with the previously reported results from NMR experiments, magnetic susceptibility measurements and other crystallographic determinations on different metal adducts [1], [2], [3], provide an updated model for the iron processing by ferritin.

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Structural comparison of the milk-clotting enzymes bovine and camel chymosin

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Chymosin is an aspartic protease that clots milk, thus initiating cheese formation. Cows are the major source for milk, but contrary to what would be expected, bovine chymosin is not the best milk-clotting enzyme known – camel chymosin is better[1]! This surprising observation initiated this project to determine the cause of this behaviour.

The camel chymosin obtained from expression in *Aspergillus niger* has been examined. Six variants have been separated using hydrophobic interaction chromatography. The variants differ with respect to glycosylation, activity, and other, at this point, unknown properties.

The structure of the double glycosylated camel chymosin has been solved. A good comparison with bovine chymosin requires the availability of a good experimental data set. However, models but no experimental data have been deposited for the previously available structures. Therefore the structure of bovine chymosin has been determined to a higher resolution (1.8 Å) than those previously available.

The overall fold of bovine and camel chymosin is similar, however camel chymosin is found in a self-inhibited state, in which the N-terminal blocks the binding cleft. The conformation commonly seen in other aspartic proteases appears to be destabilized by charge and sterical differences of the N-terminal. The resuspended crystals of camel chymosin show milk-clotting activity, hence other conformations are available, in which the N-terminal is located outside the binding cleft. Chymosin's interactions with its inhibitors and substrates are being investigated. The structure of bovine chymosin in complex with the aspartic protease inhibitor pepstatin has been solved. The structural results will be discussed in relation to the differences in the milk-clotting properties of bovine and camel chymosin.

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Unveiling the substrate-bound structure of a Baeyer-Villiger Monooxygenase

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The high specificity, efficiency, and "green" properties of biocatalysts make them increasingly attractive alternatives to conventional chemical catalysts. The Baeyer-Villiger monooxygenases (BVMOs), which catalyze the synthetically useful Baeyer-Villiger oxidation reaction, are a promising class of biocatalysts. The broad substrate spectrum of these flavoproteins makes them particularly suited for use in industry, allowing them to be engineered for specific applications. These enzymes have an FAD cofactor, and use molecular oxygen and NADPH to convert a ketone to an ester. In spite of a great deal of research characterizing these enzymes, most notably cyclohexanone monooxygenase (CHMO) from *Acinetobacter* sp. NCIMB 9871, very little structural information is available. Thus far, one crystal structure of phenylacetone monooxygenase in complex with the FAD cofactor [1] and two crystal structures of CHMO from *Rhodococcus* sp. HI-31 in complex with both FAD and $NADP^+$ [2] have been published. These three structures revealed some of the overall domain movements that are required for BVMOs to function. In particular, a large rotation in the NADPH-binding domain was revealed to cause the sliding in of the