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