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Keywords: hemoprotein, enzyme, x-ray

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Functional studies on disulfide bond forming proteins of *Wolbachia pipientis*

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To function correctly, proteins need to be folded into their correct three-dimensional structure. For secreted proteins, a key step is the introduction of disulfide bonds between cysteine residues: disulfide bond forming (Dsb-) proteins are crucial in this step. E. coli has one of the best-characterized Dsb machineries involving two pathways, the oxidative and the isomerase pathways. While EcDsbA introduces disulfide bonds into proteins in the oxidative pathway with the help of EcDsbB, EcDsbC corrects misfolded proteins by reshuffling in the isomerase pathway [1]. Even though E. coli demonstrates a well-established model, the Dsb-pathway for other organisms including *Wolbachia pipientis* remains unclear. *Wolbachia pipientis*, a Gram-negative -proteobacterial endosymbiont, is able to infect more than 65% of all insect species [2]. Most interestingly, it has the unique ability to alter the reproduction of its host in several ways to favour its own transmission [3]. The molecular interaction between Wolbachia and its host implies that disulfide bond proteins could be involved in folding of transmitter factors. Indeed, Wolbachia's *w*Mel genome encodes three Dsb proteins, α-DsbA1 and α-DsbA2 and an integral membrane protein α-DsbB [4]. I have biochemically characterized the two soluble Dsb proteins and the membrane protein α-DsbB. α-DsbB is predicted to be similar to EcDsbB and I have shown that it uses a similar electron pathway. I have also shown that α-DsbB needs all four cysteines and exogenous ubiquinone1 to actively oxidize α-DsbA1. However it does not oxidize α-DsbA2. The binding mode is likely to be different from that of EcDsbB to EcDsbA because α-DsbA1 lacks a hydrophobic groove. I have crystallized α-DsbB using the cubic phase method. I was also able to form a stable complex which is being used in crystallization trials. The structures of α-DsbB and the α-DsbB:α-DsbA1 complex will help to shed light on their interaction mode.

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Keywords: oxidases, membrane protein, biochemical assays

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Structure of the C-terminal domain of the MutL homolog from *N. gonorrhoeae*.

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The mismatch repair (MMR) pathway serves to maintain the integrity of the genome by removing mispaired bases from the newly synthesized strand. In *E. coli*, MutS, MutL and MutH co-ordinate to discriminate the daughter strand through a mechanism involving lack of methylation on the new strand. This facilitates the creation of a nick by MutH in the daughter strand to initiate mismatch repair. Many bacteria and eukaryotes –including humans- do not possess a homolog of MutH. Although the exact strategy for strand discrimination in these organisms is yet to be ascertained, the required nicking endonuclease activity is resident in the C-terminal domain of MutL. This activity is dependent on the integrity of a conserved metal binding motif. Unlike their eukaryotic counterparts, MutL in bacteria like *Neisseria* exist in the form of a homodimer. Even though this homodimer would possess two active sites, it still acts a nicking endonuclease. Here, we present the crystal structure of the C-terminal domain (CTD) of the MutL homolog of *Neisseria gonorrhoeae* (NgoL) determined to a resolution of 2.4 Å. The protein crystallized in the space group P2(1) with cell dimensions of a = 49.5 Å, b = 62.1 Å, c = 92.1 Å and $\alpha=\gamma=90^{\circ}$, $\beta=104.6^{\circ}$. The structure shows that the metal binding motif exists in a helical configuration and that four of the six conserved motifs in the MutL family - including the metal binding site- localize together to form a composite active site. NgoL-CTD exists in the form of an elongated inverted homodimer stabilized by a hydrophobic interface rich in leucines. The inverted arrangement places the two composite active sites in each subunit on opposite lateral sides of the homodimer. Such an arrangement raises the possibility that one of the active sites is occluded due to interaction of NgoL with other protein factors involved in MMR. The presentation of only one active site to substrate DNA will ensure that nicking of only one strand occurs to prevent inadvertent and deleterious double stranded cleavage.

Keywords: mismatch repair, mutL, homodimer

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Structure and mechanism of the diiron benzoyl coenzyme A epoxidase BoxB

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A recently elucidated coenzyme A (CoA) dependent aerobic benzoate metabolic pathway [1] uses an unprecedented chemical strategy to cope with the high resonance energy of aromates by forming the non-aromatic 2,3-epoxybenzoyl-CoA [2]. The crucial dearomatizing and epoxidizing reaction is carried out by BoxB and the two required electrons are delivered by BoxA, a NADPH dependent

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