conserved water molecule W-1, the order of preference for binding to lactoperoxidase appears to be as Br^- first, Cl^- second, SCN^- third and I^- the last. The positions of these anions in the substrate binding site are further defined in terms of subsites where Br^- is located in subsite 1, Cl^- in subsite 2, SCN^- in subsite 3 and I^- in subsite 4.

Keywords: antimicrobial, heme, oxidation

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Structure and mechanism of chloromomuconolactone dehalogenase from *Rhodococcus opacus* 1CP

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Chloroaromatic compounds are often very persistent environmental pollutants. Nevertheless numerous bacteria are able to metabolise these compounds and use them as the sole energy and carbon source. *Rhodococcus opacus* 1CP is able to degrade 3-chlorocatechol via a unique variant of the modified *ortho*-pathway. This pathway involves chloromuconolactone dehalogenase which dehalogenates the 5-chloromuconolactone to *cis*-dienelactone. The enzyme shows a high similarity to muconolactone isomerases, but is not able to catalyse the isomerisation reaction. In order to characterize the catalytic mechanism of this unusal dehalogenase, we crystallised the enzyme and subjected it to X-ray structural analysis. Datasets of up to 1.65 Å resolution were collected from two different crystal forms using synchrotron radiation. Cocrystallisation with substrate analogs yielded to crystals which are currently analysed for binding, to characterise the mechanism of dehalogenation.

Keywords: biotechnology, biocrystallography, enzyme

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L-*N*-carbamoylase structure suggests a striking case of protein evolution

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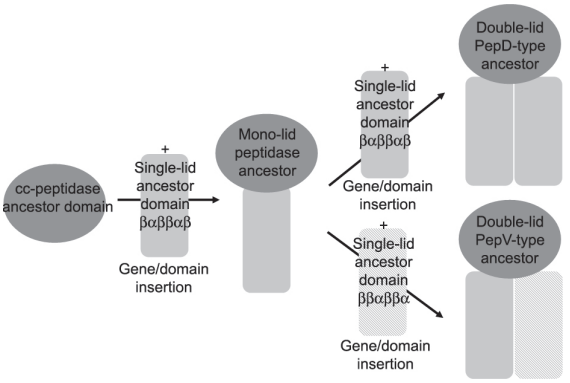
N-Carbamoyl-L-amino acid amidohydrolases (L-carbamoylases, E.C. 3.5.1.87) are important industrial enzymes used in the kinetic resolution of racemic mixtures of *N*-carbamoyl-amino acids due to their strict enantiospecificity [1]. This work reports the first L-carbamoylase structure belonging to *Geobacillus stearothermophilus* CECT43 (BsLcar) at 2.7 Å resolution showing the typical lung-shaped scaffold from the peptidase M20/M25/M40 family [2]. This common architecture is formed by two domains which for other homologs are known as catalytic and lid domains, respectively. Structural analysis of BsLcar and several members of the peptidase M20/M25/M40 family reveals several positionally conserved residues in this family, which can be hypothesized to have a critical role in substrate binding for the whole group of enzymes. An unexpected molecule bound in the catalytic cleft confirms large-scale rearrangements toward the lid domain for substrate hydrolysis, and allows us to infer the mechanism governing domain motion of L-carbamoylases. Comparative studies of the lid domain reveal a new "small molecule binding domain" (SMBD) belonging to the ACT-like superfamily. Based on BsLcar structure we propose an evolutionary pathway for all members of the peptidase M20/M25/M40 family through domain fusion and convergent/divergent evolution from common peptidase and SMBD ancestors.



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Keywords: peptidase, evolution, amidohydrolase

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Crystal structures of cytochrome c peroxidase from *Pseudomonas stutzeri* in active and inactive forms.

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Bacterial di-heme Cytochrome *c* Peroxidase (CCP) is essential to maintain H_2O_2 below toxic levels by catalyzing its reduction to H_2O. Bacterial CCP consists of two heme domains, one harboring the electron transfer heme (E heme) and the other the peroxidatic heme (P heme). The crystal structure of the CCP from *Pseudomonas stutzeri* was obtained in two different redox states. The oxidized form (inactive) crystal structure was refined to 1.6 Å resolution[1]. In this structure the peroxidatic heme is coordinated to six ligands. The reduced form, in the active mixed valence state, was refined to 2.02 Å resolution and has a water molecule bound to the peroxidatic heme. These two structures have significant conformational differences in some regions, in particular around the P heme and the interface between the two domains. Previous studies indicate that CCP from *P. stutzeri* has a very high affinity for calcium [2]. This property has been addressed from a structural point of view. Structural data will also be obtained for the CCP from the same organism in the calcium free state in the oxidized form. These structures, along with the IN and OUT forms of *P. Nautica* [3] will help to obtain a better understanding of the electron transfer mechanism within di-heme CCP and also of the role of the calcium in its activation and mechanism.

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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