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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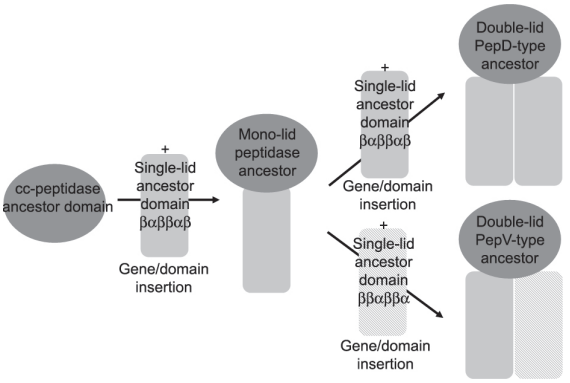
N-Carbamoyl-L-amino acid amidohydrolases (L-carbamoylases, E.C. 3.5.1.87) are important industrial enzymes used in the kinetic resolution of racemic mixtures of *N*-carbamoyl-amino acids due to their strict enantiospecificity [1]. This work reports the first L-carbamoylase structure belonging to *Geobacillus 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.