MS5-P20 The active site structure of manganese-containing Brassica rapa auxin-amidohydrolase BrILL2

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Auxin-amidohydrolase from *Brassica rapa* (*Br*), BrILL2, belongs to the M20D metallopeptidase subfamily, related to the amidohydrolase superfamily (M20) of enzymes which hydrolyze a number of different substrates, including amino acids, sugars, nucleic acids, and organophosphate esters.[1] BrILL2 is catalytically the most efficient auxin-amidohydrolase from *Br*, playing a key role in homeostasis of the plant hormone auxin in a way to hydrolases amino acid conjugates (AACs) of auxins (IAA, IBA, IPA). A large concentration of free auxins, of which the most common is indole-3-acetic acid (IAA), is toxic for plants, so only about 5% of the total concentration of auxin molecules in plants is in the free (active) form, while the rest is stored in inactive forms, mostly as amino acid and sugar conjugates.[2] In order to hydrolyze the amide bond of amino acid conjugated auxins (inactive, storage forms), and release the free auxin, BrILL needs manganese.[3] The aim of our research was to determine number of the manganese ions, Mn^{2+}, in the enzyme active site, and the influence of Cys to Ser mutations on the protein structure and activity. In order to fulfil this aim we conducted an interdisciplinary study combining different experimental and computational approaches: biochemical, spectroscopic, calorimetric and computational.

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Keywords: auxin-amidohydrolase, Brassica rapa, manganese ions, Cys, interdisciplinary study

MS5-P21 The active center and beyond: towards new selective inhibitors of *S*-adenosyl-L-homocysteine hydrolase from *Pseudomonas aeruginosa*

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Drug-resistant bacteria are an important healthy issue, as many human pathogens have gained resistance to a wide range of commercially available pharmaceuticals. *Pseudomonas aeruginosa* has a natural resistance to many antibiotics and disinfectants. This opportunistic pathogen causes a myriad of infection to immunosuppressed patients, including endocarditis, microbial keratitis of eye, pneumonia, urinary tract infections and chronic lung infection in patient with cystic fibrosis or cancer. Numerous virulence factors, the large number of multi-drug efflux systems, as well as the low permeability of the outer membrane are key factors in pathogenesis of *P. aeruginosa*. Moreover, *P. aeruginosa* is capable of growing in complex bacterial communities, called biofilms, which makes them more resistant to antibiotics than single-grown cells.

S-adenosyl-L-homocysteine hydrolase (SAHase) is an essential enzyme involved in the regulation of methylation reactions. This applies to both, healthy host cells and their invading pathogens form. Therefore, selective inhibition of SAHases in targeted cells is an excellent possibility for a drug intervention at the molecular level of cell metabolism. SAHases are highly-conserved enzymes with almost identical organization of the active site. This fact practically precludes design of highly selective inhibitors against the enzymes of pathogenic origin that would not affect the human cells. Therefore, the aim of this study is not to focus on the active site but to elucidate mechanisms of substrate and inhibitor delivery to the substrate-binding pocket of *P. aeruginosa* SAHase. The premises about various mechanisms which regulate the accessibility of the substrate binding pocked are based on crystallographic studies of SAHases from various organisms. However, the chemical nature of the different regulation mechanisms has not yet been explained. Additionally, apart from the active site, a role of a non-conservative entrance to the substrate-binding pocked in substrate delivery to the active site is proposed.

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