MS06-1-9 Tale of pesticide removal: atrazine degradation by hydroxyatrazine ethylaminohydrolase (AtzB) #MS06-1-9

A. Sharma [1], P. Kesari [1], P. Bhaumik [1]
[1]Indian Institute of Technology Bombay - Mumbai (India)

Abstract
Atrazine is a triazine ring containing herbicide introduced in the 1950s, applied to control weeds in the agricultural fields. Repeated applications and misuse of atrazine has polluted soil and surface water leading to adverse effects on humans and animals (Kumar et al. 2014). However, extensive studies have been done on atrazine degradation (Sharma et al., 2019) for more than a decade. Multiple pathways have been deduced, of which the product hydroxyatrazine is more recalcitrant and persistent in nature. Hitherto, hydroxyatrazine ethylaminohydrolase (AtzB) is the only enzyme reported that catalyses the deamination reaction of hydroxyatrazine and thus is the most important enzyme involved in atrazine degradation. Interestingly, it can mediate dechlorination reactions as well. AtzB belong to amidohydrolase family that generally catalyses hydrolysis of amidine bonds. Sequence analysis and experimental evidences reveal that AtzB is a metalloezyme containing a Zn(II) ion at the active site. However, the molecular details of the active site and catalytic mechanism of the enzyme is not known yet. Sequence optimized synthetic AtzB gene from *Pseudomonas* sp. was cloned, expressed and purified in recombinanat form. The pH optimum of AtzB ranges between 6.5-7.0. Kinetic studies show that AtzB activity is inhibited at high hydroxyatrazine (substrate) concentration and the K_m was found to be 33 μM. Structural alignment of our modelled AtzB with other homologous proteins show conservation of the Zn-coordinating residues (His74, His76, His245 and Asp331) and the catalytic active site residues comprising of Asp331, His280 and Glu248. Site directed mutations of the active site residues resulted in complete loss of activity implying their importance for AtzB function. Docking and molecular dynamics (MD) simulation studies indicated the structural features responsible for the accommodation of multiple substrates at the active site of AtzB. Based on our results we also propose a novel catalytic mechanism of AtzB and such mechanism has not been observed for other amidohydrolases.

References
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