Asp120 is not a proton donor in di-zinc *Bacillus cereus* metallo-β-lactamase

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Metallo-β-lactamases (MBL's) are enzymes with a zinc binding motif in their active site. The absence of zinc in the active site renders these enzymes inactive towards different antibiotics. At present, there are no clinically useful inhibitors for them. A rational design of new inhibitors should necessarily rely on a thorough knowledge of their catalytic mechanism, still unveiled.

Asp120 is conserved in all known metallo-β-lactamases. This residue is a zinc ligand in enzymes containing two metal ions, whereas in mono zinc enzymes it is involved in a strong hydrogen bond interaction with the nucleophilic OH^-.

To evaluate the role of Asp120 in the hydrolysis of β-lactam antibiotics, we engineered four mutants of the enzyme BcII in this position. All of the mutants showed decreased activity against different β-lactam antibiotics, with the following trend: D90E > D90N, D90Q > D90S. None of the mutants was totally inactive. Solvent Kinetic Isotope Effect experiments with different β-lactam antibiotics indicate that the rate limiting step is a proton transfer, as in the wild type enzyme, even in the absence of a residue with appropriate acid/base properties. Replacement of Asp120, a ligand to one of the zinc ions in the active site, does not abolish the ability of the enzyme to bind two metal ion equivalents. Characterization of the structure of the active sites by means of electronic spectroscopy on the Co(II)-substituted derivatives indicated that a tetrahedral metal site similar to the one in wild type BcII is conserved in these mutants and indicated the presence of Co(II) coordinated to a Cys residue in a slightly distorted second site.

These results suggest that proton transfer takes place when Asp120 is replaced by a residue incapable of acid/base catalysis, but less efficiently. In dinuclear BcII, as in other dinuclear MBLs, a water molecule might be the proton donor, and Asp120 might participate in steering this water molecule for proton transfer through a conserved hydrogen bond network.