Diversity, evolution and mechanism of zinc β-lactamases

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β-lactamases represent the prevalent resistance mechanism to β-lactam antibiotics. In the last decade, the dissemination of genes coding for metallo-β-lactamases (MBL´s) has become an emergent clinical problem. MBL´s are zinc-dependent enzymes. The exponential growth of MBL sequences being characterized has revealed an initially unforeseen structural diversity, that gives rise to the presence of mono- and dinuclear metal sites. MBL´s have been recently subdivided into classes B1, B2 and B3, each of them displaying different zinc ligands and coordination geometries.

We have employed site-directed mutagenesis in the B1 MBL from *B.cereus* (BcII) to explore the catalytically active zinc coordination geometries that can be accomodated within the active site. By these means, we proposed that one Zn(II) ion plays an essential, catalytic role, while the second one is cocatalytic. Mechanistic studies in BcII also suggest a role for each Zn(II) ion in the active site. Engineering of the metal ligands present in B3 lactamases or in related hydrolases impaires the lactamase performance, but induces other hydrolytic activities.

Directed evolution was used as an evolutionary engineering tool to explore the effect of challenging MBLs towards different antibiotics. has been considered as a precursor of more efficient MBL's present in pathogenic bacteria. In vitro evolution experiments on BcII by DNA shuffling with a cephalosporin substrate resulted in a expanded substrate spectrum of this enzyme, without sacrificing its stability nor the hydrolytic efficiency towards classical substrates of BcII. The mutations that give rise to these effects parallel others naturally found in MBL´s from pathogenic bacteria, and are related to the second-shell ligands of the zinc ions, expected to play a supramolecular control of reactivity. One of these mutations influences the structure of the cocatalytic zinc ion.