Directed evolution in Metallo-b-Lactamases: Natural *vs. in vitro* fine tuning of enzyme activity

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β-lactamases represent the main resistance mechanism developed by bacteria to escape the action of β-lactam antibiotics. Metallo-β-lactamases (MBL´s) represent the latest generation of these enzymes, being able to hydrolize penicillins, cephalosporins and carbapenems. This feature has thwarted the design of a clinically useful inhibitor for these enzymes.
The MBL from *B.cereus* (BcII hereafter) has been considered as a precursor of other, more efficient lactamases, from pathogenic bacteria. In an attempt to further expand its substrate spectrum, we generated different mutants of BcII by directed evolution towards cephalexin, a poorly hydrolyzed substrate. The minimum inhibitory concentration towards cephalexin of *E.coli* cells exporting BcII to the peripasmic space was raised 64-fold. Selected clones were sequenced, and the resulting enzymes exhibited enhanced hydrolytic capabilities towards cephalosporins with small-size substituents in the 3-position, that accomodate in an open groove in the enzyme active site. This activity enhancement was not due to a better binding of the substrates, but to a stabilization of the transition state in the hydrolytic mechanism.
The most relevant mutation found in the directed evolution experiments has been already found in nature, and is related to a broadening of the substrate spectrum among plasmid-encoded MBL´s. None of these mutations occur in the active site, and two of them are remote. This could be possibly due to the existence of molecular motions in the active site coupled with remote positions in the protein structure that are able to control reactivity.